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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year)

13 January 2000 (13.01.00)

International application No.

PCT/SE99/00544

Applicant's or agent's file reference

2990100

International filing date (day/month/year)

31 March 1999 (31.03.99)

Priority date (day/month/year)

02 April 1998 (02.04.98)

Applicant

LUNDGREN-AKERLUND, Evy

1. The designated Office is hereby notified of its election made:



in the demand filed with the International Preliminary Examining Authority on:

11 October 1999 (11.10.99)



in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

R. E. Stoffel

Telephone No.: (41-22) 338.83.38

09/697544

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From the INTERNATIONAL BUREAU

NOTIFICATION OF THE RECORDING
OF A CHANGE

(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

To:

AWAPATENT AB
P.O. Box 5117
S-200 71 Malmö
SUÈDE

RECEIVED

DEC 28 2000

TECH CENTER 1600/2000

Date of mailing (day/month/year) 16 October 2000 (16.10.00)	
Applicant's or agent's file reference 2990100	IMPORTANT NOTIFICATION
International application No. PCT/SE99/00544	International filing date (day/month/year) 31 March 1999 (31.03.99)

1. The following indications appeared on record concerning: <input checked="" type="checkbox"/> the applicant <input type="checkbox"/> the inventor <input type="checkbox"/> the agent <input type="checkbox"/> the common representative		
Name and Address ACTIVE BIOTECH AB Scheelevägen 22 S-220 07 Lund Sweden	State of Nationality SE	State of Residence SE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning: <input checked="" type="checkbox"/> the person <input type="checkbox"/> the name <input type="checkbox"/> the address <input type="checkbox"/> the nationality <input type="checkbox"/> the residence		
Name and Address CARTELA AB c/o Evy Lundgren-Åkerlund Trollsjövägen 165 S-237 33 Bjärred Sweden	State of Nationality SE	State of Residence SE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
3. Further observations, if necessary:		
4. A copy of this notification has been sent to: <input checked="" type="checkbox"/> the receiving Office <input type="checkbox"/> the designated Offices concerned <input type="checkbox"/> the International Searching Authority <input checked="" type="checkbox"/> the elected Offices concerned <input type="checkbox"/> the International Preliminary Examining Authority <input type="checkbox"/> other:		

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Aino Metcalfe Telephone No.: (41-22) 338.83.38
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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PC-2990100	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/SE99/00544	International filing date (day/month/year) 31.03.1999	Priority date (day/month/year) 02.04.1999
International Patent Classification (IPC) or national classification and IPC C 07 K 14/705, A 61 K 38/17, C 07 K 16/28		
Applicant ACTIVE BIOTECH AB et al		

<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of <u>5</u> sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of <u>19</u> sheets.</p> <p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input checked="" type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input type="checkbox"/> Certain observations on the international application
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Date of submission of the demand 11.10.1999	Date of completion of this report 15.06.2000
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. 08-667 72 88	Authorized officer Patrick Andersson/gh Telephone No. 08-782 25 00

I. Basis of the report

1. This report has been drawn on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

☐ the international application as originally filed.

☒ the description, pages 1-49, as originally filed,

pages _____, filed with the demand,

pages _____, filed with the letter of _____,

pages _____, filed with the letter of _____.

☒ the claims, Nos. _____, as originally filed,

Nos. _____, as amended under Article 19,

Nos. _____, filed with the demand,

Nos. 1-134, filed with the letter of 29.05.2000,

Nos. _____, filed with the letter of _____.

☒ the drawings, sheets/fig 1-17, as originally filed,

sheets/fig _____, filed with the demand

sheets/fig _____, filed with the letter of _____,

sheets/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/fig _____

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the supplemental box (Rule 70.2(c)).

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/SE99/00544

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. _____

because:

☒ the said international application, or the said claims Nos. X)

relate to the following subject matter which does not require an international preliminary examination (specify):

X) claims 86-126 (partially) and 127-134 (completely)

Claims 86-126 (partially) and claims 127-134 (completely) relates to in vivo methods of treatment of the human or animal body by therapy/ diagnostic methods practised on the human or animal body Rule. 67.1. (iv).

This report concerns only the in vitro methods of claims 86-126.

☒ the description, claims or drawings (indicate particular elements below) or said claims Nos. X)
are so unclear that no meaningful opinion could be formed (specify):

X) claims 10-11, 18-20, 28-29, 46-53, 73-75, 78, and 99-106 (partially)

claims 10-11, 18-20, 28-29, 46-53, 78 and 99-106 (partially) relate to "binding entities" specific to $\alpha 10$ integrin or homologues or fragments thereof, claims 73-75 relate to a pharmaceutical agent having $\alpha 10$ integrin as a target molecule. In the claims the wordings "binding entities" or "pharmaceutical agent" (claim 73) are too broad to permit a statement of a meaningful opinion and they could include known substances, i.e. claims directed to these entities fails to comply with PCT-Art 6. This opinion is limited to antibodies as binding entities/pharmaceutical agents specific to $\alpha 10$ integrins.

☐ the claims, or said claims Nos. _____ are so inadequately supported
by the description that no meaningful opinion could be formed.

☐ no international search report has been established for said claims Nos. _____

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	4, 6, 9, 17, 22-30, 33-35, 48-50, 54-72, 74-78, 82-84, 88-90, 101-103, 107-126	YES
	Claims	1-3, 5, 7-8, 10-16, 18-21, 31-32, 36-47, 51-53, 73	NO
Inventive step (IS)	Claims	4, 6, 9, 17, 22-30, 33-35, 48-50, 54-78, 88-90, 101-103, 107-125	YES
	Claims	1-3, 5, 7-8, 10-16, 18-21, 31-32, 36-47, 51-53, 79-87, 91-100, 104-106, 126	NO
Industrial applicability (IA)	Claims	1-126	YES
	Claims		NO

2. Citations and explanations

The claimed invention relates to an integrin subunit $\alpha 10$ or homologues or fragments thereof having similar biological activity. The application further contains items related to $\alpha 10$ e.g. recombinant production of $\alpha 10$, isolation of $\alpha 10$ from a cell, a polynucleotide encoding $\alpha 10$, a polynucleotide complementary to a polynucleotide encoding $\alpha 10$, a vector comprising the polynucleotide encoding the $\alpha 10$, a cell comprising the vector, fragments of $\alpha 10$ and related subjects, a process for in vitro studies of differentiation of chondrocytes, a pharmaceutical composition comprising an antibody using $\alpha 10$ as a target molecule, a vaccine comprising $\alpha 10$ and in vitro methods for detecting or studying $\alpha 10$ or its binding entities.

The following documents are considered relevant:

D1) WO92/19647

D2) Camper L, "Integrin $\alpha 2 \beta 1$ Is a receptor for the cartilage Matrix Protein Chondroadherin", 1997, vol 138, page 1159, Journal of Cell Biology

D3) WO94/25487

D4) Takada Y et al. "Molecular cloning and expression of the cDNA for alpha 3 subunit of human alpha 3 beta 1 (VLA-3), an integrin receptor for fibronectin, laminin, and collagen", 1991, Medline accession no. 92011866 & vol 115, p257-66 J Cell Biol

D5) Takada Y et al., "The primary structure of the alpha 4 subunit of VLA-4: homology to other integrins and a possible cell-cell adhesion function", 1989, medlin accession no. 89356603 & vol 8, page 1361-8,

D6) WO97/31653, not in the search report, disclosed

.../...

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

D1-D6 disclose collagen binding integrins, isolation or recombinant production of α -integrins, antibodies directed towards α -integrin, its use in pharmaceutical compositions and the use of the antibodies to detect α -integrins as markers for various conditions in e.g. chondrocytes. None of D1-D6 disclose an integrin subunit comprising essentially of the amino acid sequence in SEQ ID No 1 or fragments thereof. However, in the absence of a definition of the biological activity in the wording "essentially the same biological activity" of the $\alpha 10$ integrin, any α -integrin could be regarded to have essentially the same biological activity and consequently may be regarded as a potential homologue (i.e. having similar gene structure indicating a common evolutionary origin and/or having similar function).

Consequently, claims 1-3, 5, 7-8, 10-16, 18-21, 31-32, 36-47, 51-53, 85-87, 91-100, 104-106 lacks novelty.

In view of D1-D6 it seems obvious to a person skilled in the art to use integrins as markers/targets for different states in chondrocytes or other cells. Thus the invention according to claims 77-78, 82-84 and 126 may be novel but is not considered to involve an inventive step.

The invention according to claims 4, 6, 9, 17, 22-30, 33-35, 48-50, 54-72, 88-90, 101-103, 107-125 is considered to be novel, industrially applicable and to involve an inventive step.

D3 disclose a method for studying interactions of integrins. Thus, the invention according to claims 79-81 lacks novelty.

D6 discloses pharmaceutical compositions comprising antibodies directed against integrins or modulators of integrin expression. D6 concerns the treatment of breast cancer it is considered obvious to use the concept for other conditions depending on the function of the integrin. Integrins are involved in cartilage homeostasis (see e.g. D2); consequently, the concept of D6 can be applied for blocking cartilage formation after e.g. transplantation. Thus, the invention according to claims 73 and 86 lacks novelty; the invention according to claims 74-76 may be novel, but is not considered to involve an inventive step.

CLAIMS

1. A recombinant or isolated collagen binding integrin subunit $\alpha 10$ comprising essentially the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or homologues or fragments thereof having essentially the same biological activity.
2. A process of producing a recombinant integrin subunit $\alpha 10$ comprising essentially the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or homologues or fragments thereof having essentially the same biological activity, which process comprises the steps of
 - a) isolating a polynucleotide comprising a nucleotide sequence coding for an integrin subunit $\alpha 10$, or homologues or fragments thereof having essentially the same biological activity,
 - b) constructing an expression vector comprising the isolated polynucleotide,
 - c) transforming a host cell with said expression vector,
 - d) culturing said transformed host cell in a culture medium under conditions suitable for expression of integrin subunit $\alpha 10$, or homologues or fragments thereof having essentially the same biological activity, in said transformed host cell, and, optionally,
 - e) isolating the integrin subunit $\alpha 10$, or homologues or fragments thereof having essentially the same biological activity, from said transformed host cell or said culture medium.
3. A process of providing an integrin subunit $\alpha 10$, or homologues or fragments thereof having essentially the same biological activity, whereby said subunit is isolated from a cell in which it is naturally present.
4. An isolated polynucleotide comprising a nucleotide coding for an integrin subunit $\alpha 10$, or for homologues or fragments thereof having essentially the same

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biological activity, which polynucleotide comprises essentially the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2 or suitable parts thereof.

5 An isolated polynucleotide or oligonucleotide which hybridises to a DNA or RNA coding for an integrin subunit $\alpha 10$, or for homologues or fragments thereof having essentially the same biological activity, wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit
10 $\alpha 1$.

6. A vector comprising a polynucleotide or oligonucleotide coding for an integrin subunit $\alpha 10$, or for homologues or fragments thereof having essentially the same biological activity, which polynucleotide or oligonucleotide comprises essentially the nucleotide
15 sequence shown in SEQ ID No. 1 or SEQ ID No. 2 or parts thereof.

7. A vector comprising a polynucleotide or oligonucleotide which hybridises to a DNA or RNA coding for an integrin subunit $\alpha 10$, or for homologues or fragments thereof, wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit $\alpha 1$.
20

8. A cell containing the vector as defined in any
25 one of claims 6 and 7.

9. A cell generated by steps a) to d) of the process as defined in claim 2, in which a polynucleotide or oligonucleotide coding for an integrin subunit $\alpha 10$, or for homologues or fragments thereof having essentially
30 the same biological activity, which polynucleotide or oligonucleotide comprises the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2 or parts thereof, has been stably integrated in the cell genome.

10. Binding entities having the capability of binding specifically to an integrin subunit $\alpha 10$ comprising
35 the amino acid sequence of SEQ ID No. 1 or SEQ ID No. 2, or to homologues or fragments thereof.

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11. Binding entities according to claim 10, which are chosen from the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, and fragments thereof.

5 12. Binding entities according to claim 10, which are polyclonal or monoclonal antibodies, or fragments thereof.

10 13. A recombinant or isolated integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , in which the subunit $\alpha 10$ comprises essentially the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, and homologues and fragments thereof having essentially the same biological activity.

15 14. A recombinant or isolated integrin heterodimer according to claim 13, wherein the subunit β is $\beta 1$.

20 15. A process of producing a recombinant integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , in which the subunit $\alpha 10$ comprises essentially the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, and homologues and fragments thereof having essentially the same biological activity, which process comprises the steps of

25 a) isolating one polynucleotide comprising a nucleotide sequence coding for a subunit $\alpha 10$ of an integrin heterodimer and, optionally, another polynucleotide comprising a nucleotide sequence coding for a subunit β of an integrin heterodimer, or polynucleotides or oligonucleotides coding for homologues or fragments thereof having essentially the same biological activity,

30 b) constructing an expression vector comprising said isolated polynucleotide coding for said subunit $\alpha 10$ optionally in combination with an expression vector comprising said isolated nucleotide coding for said subunit β ,

35 c) transforming a host cell with said expression vector or vectors,

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d) culturing said transformed host cell in a culture medium under conditions suitable for expression of an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or homologues or fragments thereof having essentially the same biological activity, in said transformed host cell, and, optionally,

e) isolating the integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or homologues or fragments thereof having essentially the same biological activity, or the $\alpha 10$ subunit thereof from said transformed host cell or said culture medium.

16. A process of providing a integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or homologues or fragments thereof having essentially the same biological activity, whereby said integrin heterodimer is isolated from a cell in which it is naturally present.

17. A cell containing a first vector, said first vector comprising a polynucleotide or oligonucleotide coding for a subunit $\alpha 10$ of an integrin heterodimer, or for homologues or parts thereof having essentially the same biological activity, which polynucleotide or oligonucleotide comprises essentially the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2 or parts thereof, and a second vector, said second vector comprising a polynucleotide or oligonucleotide coding for a subunit β of an integrin heterodimer, or for homologues or fragments thereof having essentially the same biological activity.

18. Binding entities having the capability of binding specifically to an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or to homologues or fragments thereof having essentially the same biological activity, or an subunit $\alpha 10$ thereof, having essentially the same biological activity.

19. Binding entities according to claim 18, wherein the subunit β is $\beta 1$.

20. Binding entities according to claim 18 or 19, which are chosen among the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, and fragments thereof.

5 21. Binding entities according to claim 18 or 19, which are polyclonal or monoclonal antibodies

22. A fragment of the integrin subunit $\alpha 10$, which fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the
10 spliced domain.

23. A fragment according to claim 22, which is a peptide comprising the amino acid sequence
KLGFFAHKKIPEEEKREEKLEQ.

24. A fragment according to claim 22, which com-
15 prises the amino acid sequence from about amino acid No. 952 to about amino acid no. 986 of SEQ ID No. 1.

25. A fragment according to claim 22, which is a peptide comprising the amino acid sequence from about amino acid No. 140 to about amino acid no. 337 of
20 SEQ ID No. 1.

26. A method of producing a fragment of the integrin subunit $\alpha 10$ as defined in any one of claims 22-25, which method comprises a sequential addition of amino acids containing protective groups.

25 27. A polynucleotide or oligonucleotide coding for a fragment of the integrin subunit $\alpha 10$ as defined in any one of claims 22-25.

28. Binding entities having the capability of binding specifically to a fragment of the human integrin sub-
30 unit $\alpha 10$ as defined in any one of claims 22-25.

29. Binding entities according to claim 28, which are chosen from the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, and fragments thereof.

35 30. Binding entities according to claim 28, which are polyclonal or monoclonal antibodies, or fragments thereof.

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31. An *in vitro* process of using an integrin subunit $\alpha 10$ comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit $\alpha 10$ and a subunit β , or a
5 homologue or fragment of said integrin or subunit having essentially the same biologically activity, as a marker or target molecule of cells or tissues expressing said integrin subunit $\alpha 10$, which cells or tissues are of animal including human origin.
- 10 32. An *in vitro* process according to claim 31, whereby said fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.
- 15 33. An *in vitro* process according to claim 31, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.
- 20 34. An *in vitro* process according to claim 31, whereby said fragment comprises the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.
- 25 35. An *in vitro* process according to claim 31, whereby said fragment comprises the amino acid sequence from about amino acid no. 140 to about amino acid no. 337 of SEQ ID No. 1.
- 30 36. An *in vitro* process according to claim 31, whereby the subunit β is $\beta 1$.
37. An *in vitro* process according to claim 31, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.
38. An *in vitro* process according to any one of claims 31-37, which process is used during pathological conditions involving said subunit $\alpha 10$.
- 35 39. An *in vitro* process according to claim 38, which pathological conditions comprise damage of cartilage.

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40. An *in vitro* process according to claim 38, which pathological conditions comprise trauma, rheumatoid arthritis and osteoarthritis.

5 41. An *in vitro* process according to any one of claims 31-37, which is a process for detecting the formation of cartilage during embryonal development.

42. An *in vitro* process according to any one of claims 31-37, which is a process for detecting physiological or therapeutic reparation of cartilage.

10 43. An *in vitro* process according to any one of claims 31-37, which is a process for selection and analysis, or for sorting, isolating or purification of chondrocytes.

15 44. An *in vitro* process according to any one of claims 31-37, which is a process for detecting regeneration of cartilage or chondrocytes during transplantation of cartilage or chondrocytes.

20 45. A process according to any one of claims 31-37, which is a process for *in vitro* studies of differentiation of chondrocytes.

25 46. An *in vitro* process of using binding entities having the capability of binding specifically to an integrin subunit $\alpha 10$ comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit $\alpha 10$ and a subunit β , or to homologues or fragments thereof having essentially the same biological activity, as markers or target molecules of cells or tissues expressing said integrin subunit $\alpha 10$, which cells or tissues are of animal
30 including human origin.

47. An *in vitro* process according to claim 46, whereby said fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

35 48. An *in vitro* process according to claim 46, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEBEKREEKLEQ.

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49. An *in vitro* process according to claim 46, whereby said fragment comprises the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

5 50. An *in vitro* process according to claim 46, whereby said fragment comprises the amino acid sequence from about amino acid no. 140 to about amino acid No. 337 of SEQ ID No. 1.

10 51. An *in vitro* process according to claim 46, whereby the subunit β is β_1 .

52. An *in vitro* process according to any one of claims 46-51, which is a process for detecting the presence of an integrin subunit α_{10} comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or
15 of an integrin heterodimer comprising said subunit α_{10} and a subunit β , or of homologues or fragments thereof having essentially the same biological activity.

53. An *in vitro* process according to any one of claims 46-51, which process is a process for determining
20 the differentiation-state of cells during embryonic development, angiogenesis, or development of cancer.

54. An *in vitro* process for detecting the presence of a integrin subunit α_{10} , or of a homologue or fragment of said integrin subunit having essentially the same
25 biological activity, on cells, whereby a polynucleotide or oligonucleotide chosen from the group comprising a polynucleotide or oligonucleotide shown in SEQ ID No. 1 is used as a marker under hybridisation conditions wherein said polynucleotide or oligonucleotide fails to
30 hybridise to a DNA or RNA encoding an integrin subunit α_1 .

55. An *in vitro* process according to claim 54, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells,
35 osteoblasts and fibroblasts.

56. An *in vitro* process according to claim 54, whereby said fragment is a peptide chosen from the group

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comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

57. An *in vitro* process according to claim 54, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

58. An *in vitro* process according to claim 54, whereby said fragment comprises the amino acid sequence from about amino acid No. 952 to about amino acid no. 986 of SEQ ID No. 1.

59. An *in vitro* process according to claim 54, whereby said fragment comprises the amino acid sequence from about amino acid No. 140 to about amino acid No. 337 of SEQ ID No. 1.

60. An *in vitro* process according to any one of claims 54-59, which is a process for determining the differentiation-state of cells during development, in pathological conditions, in tissue regeneration or in therapeutic and physiological reparation of cartilage.

61. An *in vitro* process according to claim 60, wherein the pathological conditions are any pathological conditions involving the integrin subunit $\alpha 10$.

62. An *in vitro* process according to claim 61, whereby said pathological conditions are rheumatoid arthritis, osteoarthritis or cancer.

63. An *in vitro* process according to claim 60, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

64. An *in vitro* process for determining the differentiation-state of cells during development, in pathological conditions, in tissue regeneration and in therapeutic and physiological reparation of cartilage, whereby a polynucleotide or oligonucleotide chosen from the nucleotide sequence shown in SEQ ID No. 1 is used as a marker under hybridisation conditions wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit $\alpha 1$.

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65. An *in vitro* process according to claim 64,
whereby said polynucleotide or oligonucleotide is a
polynucleotide or oligonucleotide coding for a peptide
chosen from the group comprising peptides of the
5 cytoplasmic domain, the I-domain and the spliced domain.

66. An *in vitro* process according to claim 65,
whereby said polynucleotide or oligonucleotide is a
polynucleotide or oligonucleotide coding for a peptide
comprising the amino acid sequence
10 KLGFFAHKKIPEEEKREEKLEQ.

67. An *in vitro* process according to claim 65,
whereby said peptide comprises the amino acid sequence
from about amino acid no. 952 to about amino acid no. 986
of SEQ ID No. 1.

68. An *in vitro* process according to claim 65,
whereby said peptide comprises the amino acid sequence
from about amino acid no. 140 to about amino acid no. 337
of SEQ ID No. 1.

69. An *in vitro* process according to claim 65,
whereby said pathological conditions are any pathological
conditions involving the integrin subunit $\alpha 10$.

70. An *in vitro* process according to claim 69,
whereby said pathological conditions are rheumatoid
arthritis, osteoarthritis or cancer.

71. An *in vitro* process according to claim 69,
whereby said pathological conditions are atherosclerosis
or inflammation.

72. An *in vitro* process according to any one of
claims 64-71, whereby said cells are chosen from the
30 group comprising chondrocytes, smooth muscle cells,
endothelial cells, osteoblasts and fibroblasts.

73. A pharmaceutical composition comprising as an
active ingredient a pharmaceutical agent or an antibody
which is capable of using an integrin heterodimer com-
prising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$
35 thereof, or a homologue or fragment of said integrin or

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subunit $\alpha 10$ having essentially the same biological activity, as a target molecule.

74. A pharmaceutical composition according to claim 73, for use in stimulating, inhibiting or blocking the formation of cartilage, bone or blood vessels.

75. A pharmaceutical composition according to claim 73, for use in preventing adhesion between tendon/ligaments and the surrounding tissue after infection, inflammation and after surgical intervention where adhesion impairs the function of the tissue.

76. A vaccine comprising as an active ingredient an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or subunit $\alpha 10$, or DNA or RNA coding for said integrin subunit $\alpha 10$.

77. In vitro use of the integrin subunit $\alpha 10$ as a marker or target in transplantation of cartilage or chondrocytes.

78. An in vitro method of using binding entities having the capability of binding specifically to an integrin subunit $\alpha 10$ comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit $\alpha 10$ and a subunit β , or to homologues or fragments thereof having essentially the same biological activity, for promoting adhesion of chondrocytes and/or osteoblasts to surfaces of implants to stimulate osseointegration.

79. A method of in vitro detecting the presence of integrin binding entities, comprising interaction of an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or subunit having essentially the same biological activity, with a sample, thereby causing said integrin, subunit $\alpha 10$, or homologue or fragment thereof, to modulate the binding to its natural ligand or other integrin binding proteins present in said sample.

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80. A method of *in vitro* studying consequences of the interaction of a human heterodimer integrin comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or subunit having essentially the same biological activity, with an integrin binding entity and thereby initiate a cellular reaction.

81. A method according to claim 80, whereby the consequences of said interactions are measured as alterations in cellular functions.

82. An *in vitro* method of using DNA or RNA encoding an integrin subunit $\alpha 10$ or homologues or fragments thereof as a target molecule.

83. An *in vitro* method according to claim 82, whereby a polynucleotide or oligonucleotide hybridises to the DNA or RNA encoding an integrin subunit $\alpha 10$, or homologues or fragments thereof having essentially the same biological activity, and whereby said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit $\alpha 1$.

84. An *in vitro* method of using a human heterodimer integrin comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or subunit, or a DNA or RNA encoding an integrin subunit $\alpha 10$ or homologues or fragments thereof, as a marker or target molecule during angiogenesis.

85. A pharmaceutical composition comprising as an active ingredient a pharmaceutical agent or an antibody which is capable of stimulating cell surface expression of an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or subunit $\alpha 10$ having essentially the same biological activity.

86. A process of using a collagen binding integrin subunit $\alpha 10$ comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit $\alpha 10$ and a subunit β , or a

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homologue or fragment of said integrin or subunit having essentially the same biological activity, as a marker or target molecule of cells or tissues expressing said integrin subunit $\alpha 10$, which cells or tissues are of animal including human origin.

87. A process according to claim 86, whereby said fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

88. A process according to claim 86, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

89. A process according to claim 86, whereby said fragment comprises the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

90. A process according to claim 86, whereby said fragment comprises the amino acid sequence from about amino acid no. 140 to about amino acid no. 337 of SEQ ID No. 1.

91. A process according to claim 86, whereby the subunit β is $\beta 1$.

92. A process according to claim 86, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

93. A process according to any one of claims 86-92, which process is used during pathological conditions involving said subunit $\alpha 10$.

94. A process according to claim 93, which pathological conditions comprise damage of cartilage.

95. A process according to claim 93, which pathological conditions comprise trauma, rheumatoid arthritis and osteoarthritis.

96. A process according to any one of claims 86-92, which is a process for detecting the formation of cartilage during embryonal development.

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97. A process according to any one of claims 86-92, which is a process for detecting physiological or therapeutic reparation of cartilage.

5 98. A process according to any one of claims 86-92, which is a process for detecting regeneration of cartilage or chondrocytes during transplantation of cartilage or chondrocytes.

10 99. A process of using binding entities having the capability of binding specifically to an integrin subunit $\alpha 10$ comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit $\alpha 10$ and a subunit β , or to homologues or fragments thereof having essentially the same activity, as markers or target molecules of cells or
15 tissues expressing said integrin subunit $\alpha 10$, which cells or tissues are of animal including human origin.

20 100. A process according to claim 99, whereby said fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

101. A process according to claim 99, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

25 102. A process according to claim 99, whereby said fragment comprises the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

30 103. A process according to claim 99, whereby said fragment comprises the amino acid sequence from about amino acid no. 140 to about amino acid No. 337 of SEQ ID No. 1.

104. A process according to claim 99, whereby the subunit β is $\beta 1$.

35 105. A process according to any one of claims 99-104, which is a process for detecting the presence of an integrin subunit $\alpha 10$ comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or of an integrin

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heterodimer comprising said subunit $\alpha 10$ and a subunit β , or of homologues or fragments thereof having essentially the same biologically activity.

5 106. A process according to any one of claims 99-104, which process is a process for determining the differentiation-state of cells during embryonic development, angiogenesis, or development of cancer.

107. A process for detecting the presence of an integrin subunit $\alpha 10$, or of a homologue or fragment of said integrin subunit having essentially the same activity, on cells, whereby a polynucleotide or oligonucleotide chosen from the group comprising a polynucleotide or oligonucleotide shown in SEQ ID No. 1 is used as a marker under hybridisation conditions
15 wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit $\alpha 1$.

108. A process according to claim 107, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.
20

109. A process according to claim 107, whereby said fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.
25

110. A process according to claim 107, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

111. A process according to claim 107, whereby said fragment comprises the amino acid sequence from about
30 amino acid No. 952 to about amino acid no. 986 of SEQ ID No. 1.

112. A process according to claim 107, whereby said fragment comprises the amino acid sequence from about
35 amino acid No. 140 to about amino acid No. 337 of SEQ ID No. 1.

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113. A process according to any one of claims 107-112, which is a process for determining the differentiation-state of cells during development, in pathological conditions, in tissue regeneration or in therapeutic and physiological reparation of cartilage.

114. A process according to claim 113, wherein the pathological conditions are any pathological conditions involving the integrin subunit $\alpha 10$.

115. A process according to claim 113, whereby said pathological conditions are rheumatoid arthritis, osteoarthrosis or cancer.

116. A process according to claim 113, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

117. A process for determining the differentiation-state of cells during development, in pathological conditions, in tissue regeneration and in therapeutic and physiological reparation of cartilage, whereby a polynucleotide or oligonucleotide chosen from the nucleotide sequence shown in SEQ ID No. 1 is used as a marker under hybridisation conditions wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit $\alpha 1$.

118. A process according to claim 117, whereby said polynucleotide or oligonucleotide is a polynucleotide or oligonucleotide coding for a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

119. A process according to claim 117, whereby said polynucleotide or oligonucleotide is a polynucleotide or oligonucleotide coding for a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

120. A process according to claim 117, whereby said polynucleotide or oligonucleotide is a polynucleotide or oligonucleotide coding for a peptide comprising the amino

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acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

121. A process according to claim 117, whereby said polynucleotide or oligonucleotide is a polynucleotide or
5 oligonucleotide coding for a peptide comprising the amino acid sequence from about amino acid no. 140 to about amino acid no. 337 of SEQ ID No. 1.

122. A process according to claim 117, whereby said pathological conditions are any pathological conditions
10 involving the integrin subunit $\alpha 10$.

123. A process according to claim 117, whereby said pathological conditions are rheumatoid arthritis, osteoarthrosis or cancer.

124. A process according to claim 117, whereby said
15 pathological conditions are atherosclerosis or inflammation.

125. A process according to any one of claims 117-124, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial
20 cells, osteoblasts and fibroblasts.

126. A method of using an integrin subunit $\alpha 10$ as defined in claim 1 as a marker or target in transplantation of cartilage or chondrocytes.

127. A method of using binding entities having the
25 capability of binding specifically to an integrin subunit $\alpha 10$ comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit $\alpha 10$ and a subunit β , or to homologues or fragments thereof having essentially the same
30 biological activity, for promoting adhesion of chondrocytes and/or osteoblasts to surfaces of implants to stimulate osseointegration.

128. Use of an integrin heterodimer comprising an integrin subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$
35 thereof, or a homologue or fragment of said integrin or subunit $\alpha 10$ having essentially the same biological activity, as a target for anti-adhesive drugs or

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molecules in tendon, ligament, skeletal muscle or other tissues where adhesion impairs the function of the tissue.

129. A method of stimulating, inhibiting or blocking
5 the formation of cartilage or bone, comprising administration to a subject a suitable amount of a pharmaceutical agent or an antibody which is capable of using an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or
10 fragment of said integrin or subunit $\alpha 10$ having essentially the same biological activity, as a target molecule.

130. A method of preventing adhesion between tendon/
ligaments and the surrounding tissue after infection,
15 inflammation and after surgical intervention where adhesion impairs the function of the tissue, comprising administration to a subject a suitable amount of a pharmaceutical agent or an antibody which is capable of using an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or
20 fragment of said integrin or subunit $\alpha 10$ having essentially the same biological activity, as a target molecule.

131. A method of stimulating extracellular matrix
25 synthesis and repair by activation or blockage of an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or of the subunit $\alpha 10$ thereof, or of a homologue or fragment of said integrin or subunit $\alpha 10$ having essentially the same biological activity.

132. A method of using DNA or RNA encoding an integrin subunit $\alpha 10$ or homologues or fragments thereof as
30 a target molecule.

133. A method according to claim 132, whereby a polynucleotide or oligonucleotide hybridises to the DNA
35 or RNA encoding an integrin subunit $\alpha 10$ or homologues or fragments thereof and whereby said polynucleotide or oli-

gonucleotide fails to hybridise to a DNA or RNA encoding
en integrin subunit $\alpha 1$.

134. A method of using a human heterodimer integrin
comprising a subunit $\alpha 10$ and a subunit β , or the subunit
5 $\alpha 10$ thereof, or a homologue or fragment of said integrin
or subunit having essentially the same biological
activity, or a DNA or RNA encoding an integrin subunit
 $\alpha 10$ or homologues or fragments thereof, as a marker or
target molecule during angiogenesis.

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 2990100	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/SE 99/00544	International filing date (<i>day/month/year</i>) 31 March 1999	(Earliest) Priority Date (<i>day/month/year</i>) 2 April 1998
Applicant Active Biotech Ab et al		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 7 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. ☒ Certain claims were found unsearchable (See Box I).

2. ☐ Unity of invention is lacking (See Box II).

3. ☐ The international application contains disclosure of a nucleotide and/or amino acid sequence listing and the international search was carried out on the basis of the sequence listing

☐ filed with the international application.

☐ furnished by the applicant separately from the international application,

☐ but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.

☐ transcribed by this Authority.

4. With regard to the title, ☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

☐ the text is approved as submitted by the applicant.

☒ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is:

Figure No. 1

☒ as suggested by the applicant.

☐ None of the figures.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE99/00544**Box I** Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 28-41, 43-69, 74-79, 83-85
because they relate to subject matter not required to be searched by this Authority, namely:

These claims relate to either methods of treatment by therapy or diagnostic methods practised on the human or animal body, see PCT Rule 39.1(iv). Nevertheless, a search has been executed for these claims. The search has been based on the see next page
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

alleged effects of the compounds.

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

A recombinant or isolated integrin heterodimer comprising a novel subunit $\alpha 10$ in association with a subunit β is described. The $\alpha 10$ integrin may be purified from bovine chondrocytes on a collagen-type-II affinity column. The integrin or the subunit $\alpha 10$ can be used as marker or target of all types of cells, e.g. of chondrocytes, osteoblasts and fibroblasts. The integrin or subunit $\alpha 10$ thereof can be used as marker or target in different physiological or therapeutic methods. They can also be used as active ingredients in pharmaceutical compositions and vaccines.

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/705, A61K 38/17, C07K 16/28
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	J Biol Chem., Volume 273, August 1998, Lisbet Camper et al, "Isolation, Cloning, and Sequence Analysis of the Integrin Subunit alpha 10, a Beta1-associated Collagen Binding Integrin Expressed on Chondrocytes", Issue 32, page 20383 - page 20389	1-86
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X	WO 9219647 A1 (THE SCRIPPS RESEARCH INSTITUTE), 12 November 1992 (12.11.92)	1-20, 25-29, 33-44, 48-53, 57-62, 66-86
A		21-24, 30-32, 45-47, 54-56, 63-65
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☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

14 July 1999

Date of mailing of the international search report

30-07-1999

Name and mailing address of the ISA/
Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. +46 8 666 02 86

Authorized officer

Patrick Andersson/Els
Telephone No. +46 8 782 25 00

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
3 X	J Cell Biol, Volume 115, No 1, October 1991, Takada Y, Murphy et al, "Molecular cloning and expressin of the cDNA for alpha 3 subunit of human alpha 3 beta 1 (VLA-3), an integrin receptor for fibronectin, laminin, and collagen", page 257 - page 266, Medline abstract acc. no. 92011866	1-20,25-29, 33-44,48-53, 57-62,66-86
A	--	21-24,30-32, 45-47,54-56, 63-65
4 X	EMBO J, Volume 8, No 5, May 1989, Takada Y et al, "The primary structure of tha alpha 4 subunit of VLA-4: homology to other integrins and a possible cell-cell adhesion function", page 1361 - page 1368, Medline abstract Acc. no. 89356603	1-20,25-29, 33-44,48-53, 57-62,66-86
A	--	21-24,30-32, 45-47,54-56, 63-65
5 X	J.Cell Biol., Volume 138, No 5, Sept 1997, Lisbet Camper et al, "Integrin alpha2Beta1 Is a Receptor for the Cartilage Matrix Protein Chondroadherin" page 1159 - page 1167	1-20,25-29, 33-44,48-53, 57-62,66-86
A	--	21-24,30-32, 45-47,54-56, 63-65
6 X	WO 9425487 A1 (CHILDREN'S MEDICAL CENTER CORPORATION), 10 November 1994 (10.11.94), page 16 - page 21	1-19,28, 33-42,48-52, 57-61,66-86
A	--	20-27,43-47, 53-56,62-65

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 99/00544

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
7 A	EP 0330506 A2 (DANA-FARBER CANCER INSTITUTE), 30 August 1989 (30.08.89) -- -----	1-86

INTERNATIONAL SEARCH REPORT
Information on patent family members

01/06/99

International application No.

PCT/SE 99/00544

Patent document cited in search report			Publication date	Patent family member(s)		Publication date
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				US	5310874 A	10/05/94
				US	5589570 A	31/12/96

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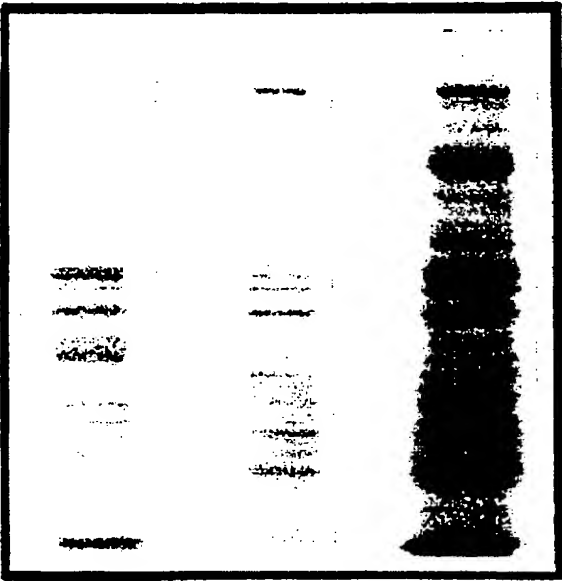
EP	0330506	A2	30/08/89	JP	2003700 A	09/01/90
				US	5583203 A	10/12/96

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/705, A61K 38/17, C07K 16/28		A1	(11) International Publication Number: WO 99/51639
			(43) International Publication Date: 14 October 1999 (14.10.99)
(21) International Application Number: PCT/SE99/00544 (22) International Filing Date: 31 March 1999 (31.03.99) (30) Priority Data: 9801164-6 2 April 1998 (02.04.98) SE 9900319-6 28 January 1999 (28.01.99) SE (71) Applicant (for all designated States except US): ACTIVE BIOTECH AB [SE/SE]; Scheelevägen 22, S-220 07 Lund (SE). (72) Inventor; and (75) Inventor/Applicant (for US only): LUND-GREN-ÅKERLUND, Evy [SE/SE]; Trollsjövägen 165, S-237 33 Bjärred (SE). (74) Agent: AWAPATENT AB; P.O. Box 5117, S-200 71 Malmö (SE).			(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: AN INTEGRIN HETERODIMER AND A SUBUNIT THEREOF			
(57) Abstract <p>A recombinant or isolated integrin heterodimer comprising a novel subunit $\alpha 10$ in association with a subunit β is described. The $\alpha 10$ integrin may be purified from bovine chondrocytes on a collagen-type-II affinity column. The integrin or the subunit $\alpha 10$ can be used as marker or target of all types of cells, e.g. of chondrocytes, osteoblasts and fibroblasts. The integrin or subunit $\alpha 10$ thereof can be used as marker or target in different physiological or therapeutic methods. They can also be used as active ingredients in pharmaceutical compositions and vaccines.</p>			
<div style="display: flex; align-items: flex-start;"><div style="margin-right: 20px;"><p>kDa</p><p>200 —</p><p>116 —</p><p>97 —</p></div><div style="text-align: center;"><p>A B C</p></div></div>			

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 99/00544

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/705, A61K 38/17, C07K 16/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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X	WO 9219647 A1 (THE SCRIPPS RESEARCH INSTITUTE), 12 November 1992 (12.11.92)	1-20,25-29, 33-44,48-53, 57-62,66-86
A		21-24,30-32, 45-47,54-56, 63-65
	--	



Further documents are listed in the continuation of Box C.



See patent family annex.

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- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

14 July 1999

Date of mailing of the international search report

30 -07- 1999

Name and mailing address of the ISA:

Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. +46 8 666 02 86

Authorized officer

Patrick Andersson/Els
Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 99/00544

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J Cell Biol, Volume 115, No 1, October 1991, Takada Y, Murphy et al, "Molecular cloning and expressin of the cDNA for alpha 3 subunit of human alpha 3 beta 1 (VLA-3), an integrin receptor for fibronectin, laminin, and collagen", page 257 - page 266, Medline abstract acc. no. 92011866	1-20,25-29, 33-44,48-53, 57-62,66-86
A	--	21-24,30-32, 45-47,54-56, 63-65
X	EMBO J, Volume 8, No 5, May 1989, Takada Y et al, "The primary structure of tha alpha 4 subunit of VLA-4: homology to other integrins and a possible cell-cell adhesion function", page 1361 - page 1368, Medline abstract Acc. no. 89356603	1-20,25-29, 33-44,48-53, 57-62,66-86
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X	J.Cell Biol., Volume 138, No 5, Sept 1997, Lisbet Camper et al, "Integrin alpha2Beta1 Is a Receptor for the Cartilage Matrix Protein Chondroadherin" page 1159 - page 1167	1-20,25-29, 33-44,48-53, 57-62,66-86
A	--	21-24,30-32, 45-47,54-56, 63-65
X	WO 9425487 A1 (CHILDREN'S MEDICAL CENTER CORPORATION), 10 November 1994 (10.11.94), page 16 - page 21	1-19,28, 33-42,48-52, 57-61,66-86
A	--	20-27,43-47, 53-56,62-65

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 99/00544

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>EP 0330506 A2 (DANA-FARBER CANCER INSTITUTE), 30 August 1989 (30.08.89)</p> <p style="text-align: center;">-- -----</p>	1-86

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE99/00544

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 28-41, 43-69, 74-79, 83-85
because they relate to subject matter not required to be searched by this Authority, namely:
These claims relate to either methods of treatment by therapy or diagnostic methods practised on the human or animal body, see PCT Rule 39.1(iv). Nevertheless, a search has been executed for these claims. The search has been based on the see next page
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE99/00544

alleged effects of the compounds.

INTERNATIONAL SEARCH REPORT
Information on patent family members

01/06/99

International application No.

PCT/SE 99/00544

Patent document cited in search report			Publication date	Patent family member(s)		Publication date
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				US	5310874 A	10/05/94
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				US	5583203 A	10/12/96

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(71) Applicant (for all designated States except US): ACTIVE BIOTECH AB [SE/SE]; Scheelevägen 22, S-220 07 Lund (SE).

(72) Inventor; and
(75) Inventor/Applicant (for US only): LUND-
GREN-ÅKERLUND, Evy [SE/SE]; Trollsjövägen
165, S-237 33 Bjärred (SE).

(74) Agent: AWAPATENT AB; P.O. Box 5117, S-200 71 Malmö (SE).

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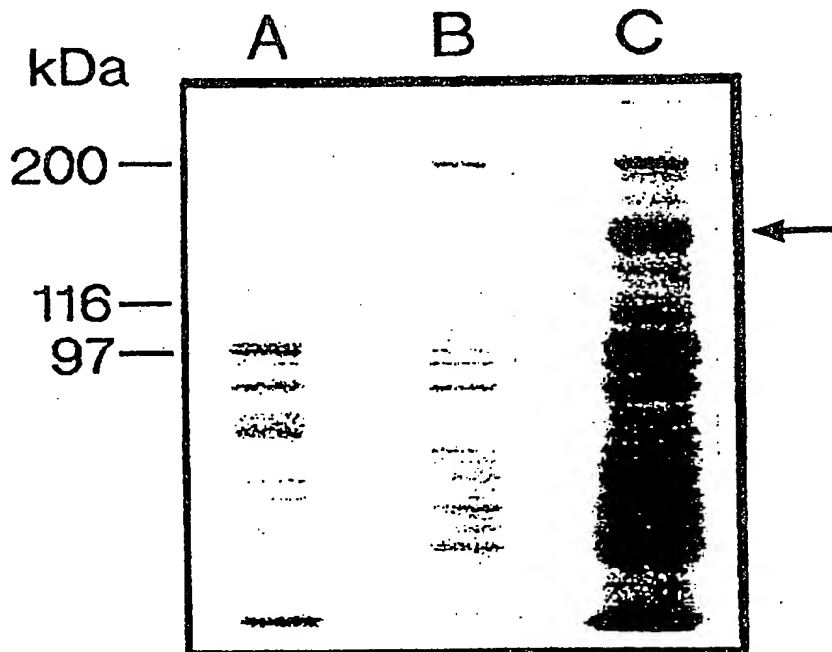
With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: AN INTEGRIN HETERODIMER AND A SUBUNIT THEREOF

(57) Abstract

A recombinant or isolated integrin heterodimer comprising a novel subunit $\alpha 10$ in association with a subunit β is described. The $\alpha 10$ integrin may be purified from bovine chondrocytes on a collagen-type-II affinity column. The integrin or the subunit $\alpha 10$ can be used as marker or target of all types of cells, e.g. of chondrocytes, osteoblasts and fibroblasts. The integrin or subunit $\alpha 10$ thereof can be used as marker or target in different physiological or therapeutic methods. They can also be used as active ingredients in pharmaceutical compositions and vaccines.



AN INTEGRIN HETERODIMER AND A SUBUNIT THEREOF

FIELD OF THE INVENTION

The present invention relates to a recombinant or isolated integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , the subunit $\alpha 10$ thereof, homologues and fragments of said integrin and of said subunit $\alpha 10$ having similar biological activity, processes of producing the same, polynucleotides and oligonucleotides encoding the same, vectors and cells comprising the same, binding entities binding specifically to the same, and the use of the same.

BACKGROUND OF THE INVENTION

The integrins are a large family of transmembrane glycoproteins that mediate cell-cell and cell-matrix interactions (1-5). All known members of this superfamily are non-covalently associated heterodimers composed of an α - and a β -subunit. At present, 8 β -subunits ($\beta 1$ - $\beta 8$) (6) and 16 α -subunits ($\alpha 1$ - $\alpha 9$, αv , αM , αL , αX , αIIb , αE and αD) have been characterized (6-21), and these subunits associate to generate more than 20 different integrins. The $\beta 1$ -subunit has been shown to associate with ten different α -subunits, $\alpha 1$ - $\alpha 9$ and αv , and to mediate interactions with extracellular matrix proteins such as collagens, laminins and fibronectin. The major collagen binding integrins are $\alpha 1\beta 1$ and $\alpha 2\beta 1$ (22-25). The integrins $\alpha 3\beta 1$ and $\alpha 9\beta 1$ have also been reported to interact with collagen (26,27) although this interaction is not well understood (28). The extracellular N-terminal regions of the α and β integrin subunits are important in the binding of ligands (29,30). The N-terminal region of the α -subunits is composed of a seven-fold repeated sequence (12,31) containing FG and GAP consensus sequences. The repeats are predicted to fold into a β -propeller domain

(32) with the last three or four repeats containing putative divalent cation binding sites. The α -integrin subunits $\alpha 1$, $\alpha 2$, αD , αE , αL , αM and αX contain a ~200 amino acid inserted domain, the I-domain (A-domain), which
5 shows similarity to sequences in von Willebrand factor, cartilage matrix protein and complement factors C2 and B (33,34). The I-domain is localized between the second and third FG-GAP repeats, it contains a metal ion-dependent adhesion site (MIDAS) and it is involved in binding of
10 ligands (35-38).

Chondrocytes, the only type of cells in cartilage, express a number of different integrins including $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 3\beta 1$, $\alpha 5\beta 1$, $\alpha 6\beta 1$, $\alpha v\beta 3$, and $\alpha v\beta 5$ (39-41). It has been shown that $\alpha 1\beta 1$ and $\alpha 2\beta 1$ mediate chondrocyte inter-
15 actions with collagen type II (25) which is one of the major components in cartilage. It has also been shown that $\alpha 2\beta 1$ is a receptor for the cartilage matrix protein chondroadherin (42).

20 SUMMARY OF THE INVENTION

The present invention relates to a novel collagen type II binding integrin, comprising a subunit $\alpha 10$ in association with a subunit β , especially subunit $\beta 1$, but also other β -subunits may be contemplated. In preferred
25 embodiments, this integrin has been isolated from human or bovine articular chondrocytes, and human chondrosarcoma cells.

The invention also encompasses integrin homologues of said integrin, isolated from other species, such as
30 bovine integrin heterodimer comprising a subunit $\alpha 10$ in association with a subunit β , preferably $\beta 1$, as well as homologues isolated from other types of human cells or from cells originating from other species.

The present invention relates in particular to a
35 recombinant or isolated integrin subunit $\alpha 10$ comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, and homologues and or fragments thereof having the

same biological activity.

The invention further relates to a process of producing a recombinant integrin subunit $\alpha 10$ comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or homologues or fragments thereof having similar biological activity, which process comprises the steps of

5 a) isolating a polynucleotide comprising a nucleotide sequence coding for a integrin subunit $\alpha 10$, or homologues or fragments thereof having similar biological activity,

10 b) constructing an expression vector comprising the isolated polynucleotide,

c) transforming a host cell with said expression vector,

15 d) culturing said transformed host cell in a culture medium under conditions suitable for expression of integrin subunit $\alpha 10$, or homologues or fragments thereof having similar biological activity, in said transformed host cell, and, optionally,

20 e) isolating the integrin subunit $\alpha 10$, or homologues or fragments thereof having the same biological activity, from said transformed host cell or said culture medium.

The integrin subunit $\alpha 10$, or homologues or fragments thereof having the same biological activity, can also be provided by isolation from a cell in which they are naturally present.

The invention also relates to an isolated polynucleotide comprising a nucleotide coding for a integrin subunit $\alpha 10$, or homologues or fragments thereof having similar biological activity, which polynucleotide comprises the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or parts thereof.

The invention further relates to an isolated polynucleotide or oligonucleotide which hybridises to a DNA or RNA encoding an integrin subunit $\alpha 10$, having the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or homologues or fragments thereof, wherein said polyoligo-

nucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding the integrin subunit $\alpha 1$.

5 The invention relates in a further aspect to vectors comprising the above polynucleotides, and to cells containing said vectors and cells that have polynucleotides or oligonucleotides as shown in SEQ ID No. 1 or 2 integrated in their genome.

10 The invention also relates to binding entities having the capability of binding specifically to the integrin subunit $\alpha 10$ or to homologues or fragments thereof, such as proteins, peptides, carbohydrates, lipids, natural ligands, polyclonal antibodies or monoclonal antibodies.

15 In a further aspect, the invention relates to a recombinant or isolated integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , in which the subunit $\alpha 10$ comprises the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or homologues or fragments thereof having similar biological activity.

20 In a preferred embodiment thereof, the subunit β is $\beta 1$.

The invention also relates to a process of producing a recombinant integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , in which the subunit $\alpha 10$ comprises
25 the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, which process comprises the steps of

a) isolating one polynucleotide comprising a nucleotide sequence coding for a subunit $\alpha 10$ of an integrin heterodimer and, optionally, another polynucleotide comprising a nucleotide sequence coding for a subunit β of
30 an integrin heterodimer, or for homologues or fragments thereof having similar biological activity,

b) constructing an expression vector comprising said isolated polynucleotide coding for said subunit $\alpha 10$ in
35 combination with an expression vector comprising said isolated nucleotide coding for said subunit β ,

c) transforming a host cell with said expression vectors,

d) culturing said transformed host cell in a culture medium under conditions suitable for expression of an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or homologues or fragments thereof similar biological activity, in said transformed host cell, and, optionally,

e) isolating the integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or homologues or fragments thereof having the same biological activity, from said transformed host cell or said culture medium.

The integrin heterodimer, or homologues or fragments thereof having similar biological activity, can also be provided by isolation from a cell in which they are naturally present.

The invention further relates to a cell containing a first vector, said first vector comprising a polynucleotide coding for a subunit $\alpha 10$ of an integrin heterodimer, or for homologues or parts thereof having similar biological activity, which polynucleotide comprises the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2 or parts thereof, and, optionally, a second vector, said second vector comprising a polynucleotide coding for a subunit β of an integrin heterodimer, or for homologues or fragments thereof.

In still another aspect, the invention relates to binding entities having the capability of binding specifically to the integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or to homologues or fragments thereof having similar biological activity, preferably wherein the subunit β is $\beta 1$. Preferred binding entities are proteins, peptides, carbohydrates, lipids, natural ligands, polyclonal antibodies and monoclonal antibodies.

In a further aspect, the invention relates to a fragment of the integrin subunit $\alpha 10$, which fragment is a peptide chosen from the group comprising peptides of

the cytoplasmic domain, the I-domain and the spliced domain.

In one embodiment, said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

5 In another embodiment, said fragment comprises the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

In a further embodiment, said fragment comprises the amino acid sequence from about amino acid No. 140
10 to about amino acid No. 337 in SEQ ID No. 1.

Another embodiment of the invention relates to a polynucleotide or oligonucleotide coding for a fragment of the human integrin subunit $\alpha 10$. In one embodiment this polynucleotide or oligonucleotide codes for a fragment
15 which is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain. In further embodiments the polynucleotide or oligonucleotide codes for the fragments defined above.

The invention also relates to binding entities having the capability of binding specifically to a fragment
20 of the integrin subunit $\alpha 10$ as defined above.

The invention also relates to a process of using an integrin subunit $\alpha 10$ comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin
25 heterodimer comprising said subunit $\alpha 10$ and a subunit β , or a homologue or fragment of said integrin or subunit having similar biological activity, as a marker or target molecule of cells or tissues expressing said integrin subunit $\alpha 10$, which cells or tissues are of animal including human origin.
30

In an embodiment of this process the fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

In further embodiments of said process the fragment
35 is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ, or a fragment comprising the amino acid sequence from about amino acid No. 952 to

about amino acid No. 986 of SEQ ID No. 1, or a fragment comprising the amino acid sequence from about amino acid no. 140 to about amino acid no. 337 of SEQ ID no. 1.

5 The subunit β is preferably $\beta 1$. The cells are preferably chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

Said process may be used during pathological conditions involving said subunit $\alpha 10$, such as pathological
10 conditions comprising damage of cartilage, or comprising trauma, rheumatoid arthritis and osteoarthritis.

Said process may be used for detecting the formation of cartilage during embryonal development, or for detecting physiological or therapeutic reparation of cartilage.

15 Said process may also be used for selection and analysis, or for sorting, isolating or purification of chondrocytes.

A further embodiment of said process is a process for detecting regeneration of cartilage or chondrocytes
20 during transplantation of cartilage or chondrocytes.

A still further embodiment of said process is a process for in vitro studies of differentiation of chondrocytes.

The invention also comprises a process of using
25 binding entities having the capability of binding specifically to an integrin subunit $\alpha 10$ comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit $\alpha 10$ and a subunit β , or to homologues or fragments thereof having
30 similar biological activity, as markers or target molecules of cells or tissues expressing said integrin subunit $\alpha 10$, which cells or tissues are of animal including human origin.

The fragment in said process may be a peptide chosen
35 from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain. In preferred embodiments said fragment is a peptide comprising the

amino acid sequence KLGFFAHKKIPEEEKREEKLEQ, or a fragment comprising the amino acid sequence from about amino acid No. 952 to about amino acid No. 986 of SEQ ID No. 1, or a fragment comprising the amino acid sequence from about amino acid No. 140 to about amino acid no. 337 of SEQ ID No. 1.

The process may also be used for detecting the presence of an integrin subunit $\alpha 10$ comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or of an integrin heterodimer comprising said subunit $\alpha 10$ and a subunit β , or of homologues or fragments thereof having similar biological activity.

In a further embodiment said process is a process for determining the differentiation-state of cells during embryonic development, angiogenesis, or development of cancer.

In a still further embodiment this process is a process for detecting the presence of an integrin subunit $\alpha 10$, or of a homologue or fragment of said integrin subunit having similar biological activity, on cells, whereby a polynucleotide or oligonucleotide chosen from the group comprising a polynucleotide or oligonucleotide chosen from the nucleotide sequence shown in SEQ ID No. 1 is used as a marker under hybridisation conditions wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit $\alpha 1$. Said cells may be chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts. Said integrin fragment may be a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain, such as a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ, or a fragment comprising the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1, or a fragment comprising the amino acid sequence from about amino acid No. 140 to about amino acid no. 337 of SEQ ID No. 1.

In a still further embodiment the process is a process for determining the differentiation-state of cells during development, in pathological conditions, in tissue regeneration or in therapeutic and physiological reparation of cartilage. The pathological conditions may be any
5 pathological conditions involving the integrin subunit $\alpha 10$, such as rheumatoid arthritis, osteoarthritis or cancer. The cells may be chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells,
10 osteoblasts and fibroblasts.

The invention also relates to a process for determining the differentiation-state of cells during development, in pathological conditions, in tissue regeneration and in therapeutic and physiological reparation of cartilage, whereby a polynucleotide or oligonucleotide
15 chosen from the nucleotide sequence shown in SEQ ID No. 1 is used as a marker under hybridisation conditions wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit
20 $\alpha 1$. Embodiments of this aspect comprise a process, whereby said polynucleotide or oligonucleotide is a polynucleotide or oligonucleotide coding for a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain,
25 such as a polynucleotide or oligonucleotide coding for a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ, or comprising the amino acid sequence from about amino acid No. 952 to about amino acid no. 986 of SEQ ID No. 1, or the amino acid sequence
30 from about amino acid No. 140 to about amino acid No. 337 of SEQ ID No. 1. Said pathological conditions may be any pathological conditions involving the integrin subunit $\alpha 10$, such as rheumatoid arthritis, osteoarthritis or cancer, or atherosclerosis or inflammation. Said cells
35 may be chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

In a further aspect the invention relates to a pharmaceutical composition comprising as an active ingredient a pharmaceutical agent or an antibody which is capable of using an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or subunit $\alpha 10$ having similar biological activity, as a target molecule. An embodiment of said pharmaceutical composition is intended for use in stimulating, inhibiting or blocking the formation of cartilage, bone or blood vessels. A further embodiment comprises a pharmaceutical composition for use in preventing adhesion between tendon/ligaments and the surrounding tissue after infection, inflammation and after surgical intervention where adhesion impairs the function of the tissue.

The invention is also related to a vaccine comprising as an active ingredient an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or subunit $\alpha 10$, or DNA or RNA coding for said integrin subunit $\alpha 10$.

A further aspect of the invention is related to the use of the integrin subunit $\alpha 10$ as defined above as a marker or target in transplantation of cartilage or chondrocytes.

A still further aspect of the invention is related to a method of using binding entities having the capability of binding specifically to an integrin subunit $\alpha 10$ comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit $\alpha 10$ and a subunit β , or to homologues or fragments thereof having similar biological activity, for promoting adhesion of chondrocytes and/or osteoblasts to surfaces of implants to stimulate osseointegration.

The invention is also related to the use of an integrin subunit $\alpha 10$ or an integrin heterodimer comprising said subunit $\alpha 10$ and a subunit β as a target for anti-

adhesive drugs or molecules in tendon, ligament, skeletal muscle or other tissues where adhesion impairs the function of the tissue.

5 The invention also relates to a method of stimulating, inhibiting or blocking the formation of cartilage or bone, comprising administration to a subject a suitable amount of a pharmaceutical agent or an antibody which is capable of using an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof,
10 or a homologue or fragment of said integrin or subunit $\alpha 10$ having similar biological activity, as a target molecule.

In another embodiment the invention is related to a method of preventing adhesion between tendon/ligaments
15 and the surrounding tissue after infection, inflammation and after surgical intervention where adhesion impairs the function of the tissue, comprising administration to a subject a suitable amount of a pharmaceutical agent or an antibody which is capable of using a integrin hetero-
20 dimer comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or subunit $\alpha 10$ having similar biological activity, as a target molecule.

The invention also relates to a method of stimulating
25 extracellular matrix synthesis and repair by activation or blockage of an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or of the subunit $\alpha 10$ thereof, or of a homologue or fragment of said integrin or subunit $\alpha 10$ having similar biological activity.

30 In a further aspect the invention relates to a method of in vitro detecting the presence of integrin binding entities, comprising interaction of an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of
35 said integrin or subunit, with a sample, thereby causing said integrin, subunit $\alpha 10$, or homologue or fragment thereof having similar biological activity, to modulate

the binding to its natural ligand or other integrin binding proteins present in said sample.

The invention also relates to a method of *in vitro* studying consequences of the interaction of a human
5 heterodimer integrin comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or subunit, with an integrin binding entity and thereby initiate a cellular reaction. Said consequences may be measured as alterations in cellular functions.
10

A still further aspect of the inventions relates to a method of using DNA or RNA encoding an integrin subunit $\alpha 10$ or homologues or fragments thereof as a molecular target. In an embodiment of this aspect, a polynucleotide
15 or oligonucleotide hybridises to the DNA or RNA encoding an integrin subunit $\alpha 10$ or homologues or fragments thereof, whereby said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit $\alpha 1$.

The invention also relates to a method of using a
20 human heterodimer integrin comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or subunit, or a DNA or RNA encoding an integrin subunit $\alpha 10$ or homologues or fragments thereof, as a marker or target molecule during
25 angiogenesis.

BRIEF DESCRIPTION OF THE FIGURES

Fig.1 Affinity purification of the $\alpha 10$ integrin subunit on collagen type II-Sepharose.

30 Fig. 2. Amino acid sequences of peptides from the bovine $\alpha 10$ integrin subunit.

Fig. 3a. Affinitypurification and immunoprecipitation of the integrin subunit $\alpha 10$ from bovine chondrocytes.

35 Fig. 3b. Affinitypurification and immunoprecipitation of the integrin subunit $\alpha 10$ from human chondrocytes.

Fig. 3c. Affinitypurification and immunoprecipitation of the integrin subunit $\alpha 10$ from human chondrosarcoma cells.

5 Fig. 4. A 900 bp PCR-fragment corresponding to the bovine integrin subunit $\alpha 10$

Fig. 5. Schematic map of the three overlapping $\alpha 10$ clones.

Fig. 6. Nucleotide sequence and deduced amino acid sequence of the human $\alpha 10$ integrin subunit.

10 Fig. 7. Northern blot of integrin $\alpha 10$ mRNA.

Fig. 8 Immunoprecipitation of the $\alpha 10$ integrin subunit from human chondrocytes using antibodies against the cytoplasmic domain of $\alpha 10$ (a). Western blot of the $\alpha 10$ associated β -chain (b).

15 Fig. 9. Immunostaining of $\alpha 10$ integrin in human articular cartilage.

Fig. 10 Immunostaining of $\alpha 10$ integrin in 3 day mouse limb cartilage.

20 Fig 11. Immunostaining of $\alpha 10$ integrin in 13.5 day mouse embryo.

Fig 12. Hybridisation of $\alpha 10$ mRNA in various human tissues.

25 Fig. 13 Immunostaining of fascia around tendon (a), skeletal muscle (b) and heart valves (c) in 3 day mouse limb.

Fig. 14. PCR fragments corresponding to $\alpha 10$ integrin subunit from human chondrocytes, human endothelial cells, human fibroblasts and rat tendon.

30 Fig 15. Partial genomic nucleotide sequence of the human integrin subunit $\alpha 10$.

Fig 16. Upregulation of $\alpha 10$ integrin subunit in chondrocytes cultured in alginate.

35 Fig 17. Immunoprecipitation of the $\alpha 10$ integrin subunit from human smooth muscle cells

DETAILED DESCRIPTION OF THE INVENTION

The present invention demonstrate that human and

bovine chondrocytes express a novel, collagen type II-binding integrin in the $\beta 1$ -family. An earlier study presented some evidence for that human chondrosarcoma cells also express this integrin (25). Immunoprecipitation experiments using antibodies against the integrin subunit $\beta 1$ revealed that this novel α -integrin subunit had an apparent molecular weight (M_r) of approximately 160 kDa under reducing conditions, and was slightly larger than the $\alpha 2$ integrin subunit. To isolate this α -subunit collagen type II-binding proteins were affinity purified from bovine chondrocytes. The chondrocyte lysate was first applied to a fibronectin-Sepharose precolumn and the flow through was then applied to a collagen type II-Sepharose column. A protein with M_r of approximately 160 kD was specifically eluted with EDTA from the collagen column but not from the fibronectin column. The M_r of this protein corresponded with the M_r of the unidentified $\beta 1$ -related integrin subunit. The 160 kD protein band was excised from the SDS-PAGE gel, digested with trypsin and the amino acid sequences of the isolated peptides were analysed.

Primers corresponding to isolated peptides amplified a 900 bp PCR-fragment from bovine cDNA which was cloned, sequenced and used for screening of a human articular chondrocyte λ ZapII cDNA library to obtain the human integrin α -subunit homologue. Two overlapping clones, hc1 and hc2 were isolated, subcloned and sequenced. These clones contained 2/3 of the nucleotide sequence including the 3' end of the cDNA. A third clone which contained the 5' end of the $\alpha 10$ cDNA, was obtained using the RACE technique. Sequence analysis of the 160 kD protein sequence showed that it was a member of the integrin α -subunit family and the protein was named $\alpha 10$.

The deduced amino acid sequence of $\alpha 10$ was found to share the general structure of the integrin α -subunits described in previously published reports (6-21). The large extracellular N-terminal part of $\alpha 10$ contains a

seven-fold repeated sequence which was recently predicted to fold into a β -propeller domain (32). The integrin subunit $\alpha 10$ contains three putative divalent cation-binding sites (DxD/NxD/NxxxD) (53), a single spanning transmembrane domain and a short cytoplasmic domain. In contrast to most α -integrin subunits the cytoplasmic domain of $\alpha 10$ does not contain the conserved sequence KxGFF (R/K) R. The predicted amino acid sequence in $\alpha 10$ is KLGFFAH. Several reports indicate that the integrin cytoplasmic domains are crucial in signal transduction (54) and that membrane-proximal regions of both α - and β -integrin cytoplasmic domains are involved in modulating conformation and affinity state of integrins (55-57). It is suggested that the GFFKR motif in α -chains are important for association of integrin subunits and for transport of the integrin to the plasma membrane (58). The KxGFFKR domain has been shown to interact with the intracellular protein calreticulin (59) and interestingly, calreticulin-null embryonic stem cells are deficient in integrin-mediated cell adhesion (60). It is therefor possible that the sequence KLGFFAH in $\alpha 10$ have a key function in regulating the affinity between $\alpha 10\beta 1$ and matrix proteins.

Integrin α subunits are known to share an overall identity of 20-40% (61). Sequence analysis showed that the $\alpha 10$ subunit is most closely related to the I-domain containing α -subunits with the highest identity to $\alpha 1$ (37%) and $\alpha 2$ (35%). The integrins $\alpha 1\beta 1$ and $\alpha 2\beta 1$ are known receptors for both collagens and laminins (24;62;63) and we have also recently demonstrated that $\alpha 2\beta 1$ interacts with the cartilage matrix protein chondroadherin (42). Since $\alpha 10\beta 1$ was isolated on a collagen type II-Sepharose, we know that collagen type II is a ligand for $\alpha 10\beta 1$. We have also shown by affinity purification experiments that $\alpha 10\beta 1$ interacts with collagen type I but it remains to be seen whether laminin or chondroadherin are also ligands for this integrin.

The $\alpha 10$ associated β -chain migrated as the $\beta 1$ integrin subunit both under reducing and non-reducing conditions. To verify that the $\alpha 10$ associated β -chain indeed is $\beta 1$, chondrocyte lysates were immunoprecipitated with
5 antibodies against $\alpha 10$ or $\beta 1$ followed by Western blot using antibodies against the $\beta 1$ -subunit. These results clearly demonstrated that $\alpha 10$ is a member of the $\beta 1$ -integrin family. However, the possibility that $\alpha 10$ combine also with other β -chains can not be excluded..

10 A polyclonal peptide antibody raised against the cytoplasmic domain of $\alpha 10$ precipitated two protein bands with M_r of approximately 160 kD ($\alpha 10$) and 125 kD ($\beta 1$) under reducing conditions. Immunohistochemistry using the $\alpha 10$ -antibody showed staining of the chondrocytes in tissue
15 sections of human articular cartilage. The antibody staining was clearly specific since preincubation of the antibody with the $\alpha 10$ -peptide completely abolished the staining. Immunohistochemical staining of mouse limb sections from embryonic tissue demonstrated that $\alpha 10$ is
20 upregulated during condensation of the mesenchyme. This indicate that the integrin subunit $\alpha 10$ is important during the formation of cartilage. In 3 day old mice $\alpha 10$ was found to be the dominating collagen binding integrin subunit which point to that $\alpha 10$ has a key function in
25 maintaining normal cartilage functions.

Expression studies on the protein and mRNA level show that the distribution of $\alpha 10$ is rather restrictive. Immunohistochemistry analyses have shown that $\alpha 10$ integrin subunit is mainly expressed in cartilage but it is
30 also found in perichondrium, periosteum, ossification groove of Ranvier, in fascia surrounding tendon and skeletal muscle and in the tendon-like structures in the heart valves. This distribution point to that $\alpha 10$ integrin subunit is present also on fibroblasts and
35 osteoblasts. PCR amplification of cDNA from different cell types revealed the presence of an alternatively spliced $\alpha 10$ integrin subunit. This spliced $\alpha 10$ was domi-

nating in fibroblasts which suggests that $\alpha 10$ in fibroblasts may have a different function compared to $\alpha 10$ present on chondrocytes.

Expression of the integrin subunit $\alpha 10$ was found to
5 decrease when chondrocytes were cultured in monolayer. In contrast, the expression of $\alpha 10$ was found to increase when the cells were cultured in alginate beads. Since the latter culturing model is known to preserve the phenotype of chondrocytes the results suggest that $\alpha 10$ can function
10 as marker for a differentiated chondrocyte.

Adhesion between tendon/ligaments and the surrounding tissue is a well-known problem after infection, injury and after surgical intervention. Adhesion between tendon and tendon sheets impairs the gliding function and
15 cause considerable problems especially during healing of tendons in e.g. the hand and fingers leading to functional incapacity. The localisation of the $\alpha 10$ integrin subunit in the fascia of tendon and skeletal muscle makes $\alpha 10$ a possible target for drugs and molecules with anti-
20 adhesive properties that could prevent impairment of the function of tendon/ligament. The integrin subunit $\alpha 10$ can also be a target for drugs or molecules with anti-adhesive properties in other tissues where adhesion is a problem.

25

EXAMPLES

Example 1

Affinity purification of the $\alpha 10$ integrin subunit on
30 collagen type II-Sepharose.

Materials and Methods

Bovine chondrocytes, human chondrocytes or human chondrosarcoma cells were isolated as described earlier [Holmvall et al, Exp Cell Res, 221, 496-503 (1995),
35 Camper et al, JBC, 273, 20383-20389 (1998)]. A Triton X-100 lysate of bovine chondrocytes was applied to a fibronectin-Sepharose precolumn followed by a collagen

type II-Sepharose column and the integrin subunit $\alpha 10$ was eluted from the collagen type II-column by EDTA (Camper et al, JBC, 273, 20383-20389 (1998). The eluted proteins were precipitated by methanol/chloroform, separated by SDS-PAGE under reducing conditions and stained with Coomassie blue. (Camper et al, JBC, 273, 20383-20389 (1998). Peptides from the $\alpha 10$ protein band were isolated by in-gel digestion with a trypsin and phase liquid chromatography and sequenced by Edman degradation (Camper et al, JBC, 273, 20383-20389 (1998).

Results

Fig 1 shows EDTA-eluted proteins from the fibronectin-Sepharose (A), flow-through from the collagen type II-Sepharose column (B) and EDTA-eluted proteins from the collagen type II-Sepharose (C). The $\alpha 10$ integrin subunit (160 kDa) which was specifically eluted from the collagen type II column is indicated with an arrow. Figure 2 shows the amino acid sequences of six peptides that were isolated from the bovine integrin subunit $\alpha 10$. Figures 3 a, b, and c show that the $\alpha 10$ integrin subunit is present on bovine chondrocytes (3a), human chondrocytes (3b) and human chondrosarcoma cells (3c). The affinity for collagen type II, the coprecipitation with $\beta 1$ -integrin subunit and the molecular weight of 160 kDa under reducing conditions identify the $\alpha 10$ integrin subunit on the different cells. These results show that $\alpha 10$ can be isolated from chondrocytes and from chondrosarcoma cells.

Example 2

Amplification of PCR fragment corresponding to bovine $\alpha 10$ integrin subunit.

Materials and methods

The degenerate primers GAY AAY ACI GCI CAR AC (DNTAQT, forward) and TIA TIS WRT GRT GIG GYT (EPHHSI; reverse) were used in PCR (Camper et al, JBC, 273, 20383-20389 (1998) to amplify the nucleotide sequence corresponding to the bovine peptide 1 (Figure 2). A 900 bp

PCR-fragment was then amplified from bovine cDNA using an internal specific primer TCA GCC TAC ATT CAG TAT (SAYIQY, forward) corresponding to the cloned nucleotide sequence of peptide 1 together with the degenerate primer ICK RTC CCA RTG ICC IGG (PGHWDR, reverse) corresponding to the bovine peptide 2 (Figure2). Mixed bases were used in positions that were twofold degenerate and inosines were used in positions that are three- or fourfold degenerate. mRNA isolation and cDNA synthesis was done as earlier described (Camper et al, JBC, 273, 20383-20389 (1998)). The purified fragment was cloned, purified and sequenced as earlier described (Camper et al, JBC, 273, 20383-20389 (1998)).

Results

The nucleotide sequence of peptide 1 (Figure 2) was obtained by PCR-amplification, cloning and sequencing of bovine cDNA. From this nucleotide sequence an exact primer was designed and applied in PCR-amplification with degenerate primers corresponding to peptides 2-6 (Figure 2). Primers corresponding to peptides 1 and 2 amplified a 900 bp PCR-fragment from bovine cDNA (Figure 4).

Example 3

Cloning and sequence analysis of the human $\alpha 10$ integrin subunit

Material and methods

The cloned 900bp PCR-fragment, corresponding to bovine $\alpha 10$ -integrin, was digoxigenin-labelled according to the DIG DNA labelling kit (Boehringer Mannheim) and used as a probe for screening of a human articular chondrocyte λ ZapII cDNA library (provided by Michael Bayliss, The Royal Veterinary Basic Sciences, London, UK) (52). Positive clones containing the pBluescript SK+ plasmid with the cDNA insert were rescued from the ZAP vector by *in vivo* excision as described in the ZAP-cDNA® synthesis kit (Stratagene). Selected plasmids were purified and

sequenced as described earlier (Camper et al, JBC, 273, 20383-20389 (1998)) using T3, T7 and internal specific primers. To obtain cDNA that encoded the 5' end of $\alpha 10$ we designed the primer AAC TCG TCT TCC AGT GCC ATT CGT GGG
5 (reverse; residue 1254-1280 in $\alpha 10$ cDNA) and used it for rapid amplification of the cDNA 5' end (RACE) as described in the Marathon™ cDNA Amplification kit (Clontech INC., Palo Alto, CA).

Results

10 Two overlapping clones, hc1 and hc2 (Figure 5), were isolated, subcloned and sequenced. These clones contained 2/3 of the nucleotide sequence including the 3' end of the cDNA. A third clone (racel; Figure 5), which contained the 5' end of the $\alpha 10$ cDNA, was obtained using the
15 RACE technique. From these three overlapping clones of $\alpha 10$ cDNA, 3884 nucleotides were sequenced. The nucleotide sequence and deduced amino acid sequence is shown in Figure 6. The sequence contains a 3504-nucleotide open reading frame that is predicted to encode a 1167 amino
20 acid mature protein. The signal peptide cleavage site is marked with an arrow, human homologues to bovine peptide sequences are underlined and the I-domain is boxed. Metal ion binding sites are indicated with a broken underline, potential N-glycosylation sites are indicated by an
25 asterisk and the putative transmembrane domain is double underlined. The normally conserved cytoplasmic sequence is indicated by a dot and dashed broken underline.

Sequence analysis demonstrate that $\alpha 10$ is a member of the integrin α -subunit family.

30

Example 4

Identification of a clone containing a splice variant of $\alpha 10$

35 One clone which was isolated from the human chondrocyte library (see Example 3) contained a sequence that was identical to the sequence of $\alpha 10$ integrin subunit except that the nucleotides between nt positions

2942 and 3055 were deleted. The splice variant of $\alpha 10$ was verified in PCR experiment using primers flanking the splice region (see figure 14).

5 Example 5

Identification of $\alpha 10$ integrin subunit by Northern blot

Material and methods

Bovine chondrocyte mRNA was purified using a
10 QuickPrep®Micro mRNA Purification Kit (Pharmacia Biotech, Uppsala, Sweden), separated on a 1% agarose-formaldehyde gel, transferred to nylon membranes and immobilised by UV crosslinking. cDNA-probes were ^{32}P -labelled with Random Primed DNA Labeling Kit (Boehringer Mannheim). Filters
15 were prehybridised for 2-4 hours at 42°C in 5x SSE, 5x Denharts solution, 0.1 % SDS, 50 µg/ml salmon sperm DNA and 50% formamide and then hybridised over night at 42 °C with the same solution containing the specific probe (0.5-1 x 10⁶ cpm/ml). Specifically bound cDNA-
20 probes were analysed using the phosphoimager system (Fuji). Filters were stripped by washing in 0.1% SDS, for 1 hour at 80°C prior to re-probing. The $\alpha 10$ -integrin cDNA-probe was isolated from the racel-containing plasmid using the restriction enzymes BamHI (GIBCO BRL) and NcoI
25 (Boehringer Mannheim). The rat $\beta 1$ -integrin cDNA probe was a kind gift from Staffan Johansson, Uppsala, Sweden.

Results

Northern blot analysis of mRNA from bovine chondrocytes showed that a human $\alpha 10$ cDNA-probe hybridised with
30 a single mRNA of approximately 5.4 kb (Figure 7). As a comparison, a cDNA-probe corresponding to the integrin subunit $\alpha 1$ was used. This cDNA-probe hybridised a mRNA-band of approximately 3.5 kb on the same filter. These results show that a cDNA-probe against $\alpha 10$ can be used to
35 identify the $\alpha 10$ integrin subunit on the mRNA level.

Example 6

Preparation of antibodies against the integrin subunit $\alpha 10$

A peptide corresponding to part of the $\alpha 10$ cytoplasmic domain, Ckkipeeeekreekle (see figure 6) was synthesised and conjugated to keyhole limpet hemocyanin (KLH). Rabbits were immunised with the peptide-KLH conjugate to generate antiserum against the integrin subunit $\alpha 10$. Antibodies recognising $\alpha 10$ were affinity purified on an peptide-coupled column (Innovagen AB).

Example 7

Immunoprecipitation of the integrin subunit $\alpha 10$ from chondrocytes

15 Material and methods

Human chondrocytes were ^{125}I -labelled¹, lyzed with Triton X-100 and immunoprecipitated as earlier described (Holmvalle et al, Exp Cell Res, 221, 496-503 (1995), Camper et al, JBC, 273, 20383-20389 (1998)). Triton X-100 lysates of ^{125}I -labeled human chondrocytes were immunoprecipitated with polyclonal antibodies against the integrin subunits $\beta 1$, $\alpha 1$, $\alpha 2$, $\alpha 3$ or $\alpha 10$. The immunoprecipitated proteins were separated by SDS-PAGE (4-12%) under non-reducing conditions and visualised using a phosphorimager. Triton X-100 lysates of human chondrocytes immunoprecipitated with $\alpha 10$ or $\beta 1$ were separated by SDS-PAGE (8%) under non-reducing conditions and analysed by Western blot using the polyclonal $\beta 1$ antibody and chemiluminescent detection as described in Camper et al, JBC, 273, 20383-20389 (1998).

Results

The polyclonal peptide antibody, raised against the cytoplasmic domain of $\alpha 10$, precipitated two protein bands with Mr of approximately 160 kD ($\alpha 10$) and 125 kD ($\beta 1$) under reducing conditions. The $\alpha 10$ associated β -chain migrated as the $\beta 1$ integrin subunit (Figure 8a). To verify that the $\alpha 10$ associated β -chain in chondrocytes

indeed is $\beta 1$, chondrocyte lysates were immunoprecipitated with antibodies against $\alpha 10$ or $\beta 1$ followed by Western blot using antibodies against the $\beta 1$ -subunit (Figure 8b). These results clearly demonstrated that $\alpha 10$ is a member of the $\beta 1$ -integrin family. However, the results do not exclude the possibility that $\alpha 10$ can associate with other β -chains in other situations.

Example 8

Immunohistochemical staining of the integrin subunit $\alpha 10$ in human and mouse cartilage

Material and methods

Frozen sections of adult cartilage (trochlear groove) obtained during surgery (provided by Anders Lindahl, Salgrenska Hospital, Gothenburg, Sweden and frozen sections from of 3 day old mouse limb were fixed and prepared for immunohistochemistry as earlier described (Camper et al, JBC, 273, 20383-20389 (1998)). Expression of $\alpha 10$ integrin subunit was analysed using the polyclonal antibody against the cytoplasmic domain as a primary antibody (see Example 6) and a secondary antibody conjugated to peroxidase.

Results

Figures 9 show immunostaining of human adult articular cartilage.

The $\alpha 10$ -antibody recognising the cytoplasmic domain of $\alpha 10$ stained the chondrocytes in tissue sections of human articular cartilage (A). The staining was depleted when the antibody was preincubated with the $\alpha 10$ - peptide (B). A control antibody recognising the $\alpha 9$ integrin subunit did not bind to the chondrocyte (C).

Figures 10 shows that the $\alpha 10$ antibody stain the majority of chondrocytes in the growing bone anlage (a and b). The $\alpha 10$ antibody also recognised cells in the ossification groove of Ranvier (b), especially the osteoblast in the bone bark which are lining the cartilage in the metaphys are highly positive for $\alpha 10$. The

cells in the ossification groove of Ranvier are believed to be important for the growth in diameter of the bone. The integrin subunit $\alpha 10$ is also highly expressed in perichondrium and periosteum. Cell in these tissues are likely important in the repair of the cartilage tissue. The described localisation of the integrin subunit $\alpha 10$ suggest that this integrin is important for the function of the cartilage tissue.

10 Example 9

Immunohistochemical staining of the integrin subunit $\alpha 10$ during mouse development

Material and methods

Frozen sections from mouse embryos (13.5 days) were investigated for expression of $\alpha 10$ by immunohistochemistry as described in Camper et al, JBC, 273, 20383-20389 (1998). Expression of $\alpha 10$ integrin subunit was analysed using the polyclonal antibody against the cytoplasmic domain as a primary antibody (see Example 6) and a secondary antibody conjugated to peroxidase. The embryo sections were also investigated for expression of integrin subunit $\alpha 1$ (monoclonal antibody from Pharmingen) and collagen type II (monoclonal antibody, kind gift from Dr John Mo, Lund University, Sweden).

25 Results

Figure 11 show that $\alpha 10$ integrin subunit is unregulated in the limb when the mesenchymal cells undergo condensation to form cartilage (a). Especially the edge of the newly formed cartilage has high expression of $\alpha 10$. The formation of cartilage is verified by the high expression of the cartilage specific collagen type II (b). The control antibody against $\alpha 1$ integrin subunit showed only weak expression on the cartilage (c). In other experiments expression of $\alpha 10$ was found in all cartilage containing tissues in the 3 day old mouse including limbs, ribs and vertebrae. The upregulation of $\alpha 10$ during formation of cartilage suggest that this integrin subunit is

important both in the development of cartilage and bone and in the repair of damaged cartilage tissue.

Example 10

5 mRNA expression of $\alpha 10$ in tissues other than articular cartilage

Material and methods

Expression of $\alpha 10$ integrin subunit was examined on the mRNA level in different human tissues. A Northern dot
10 blot with immobilised mRNA from the listed tissues in Figure 12 was hybridised with an $\alpha 10$ integrin cDNA probe isolated from the race 1-containing plasmid using the restriction enzymes *Bam*H1 and *Nco*1. The degree of hybridisation was analysed using a phospho imager. The following
15 symbols denote mRNA level in increasing order: -, +, ++, +++, +++++.

Results

Analysis of the hybridised mRNA showed that $\alpha 10$ was expressed in aorta, trachea, spinal cord, heart,
20 lung, and kidney (Figure 12). All other tissues appeared negative for $\alpha 10$ expression. These results point to a restricted distribution of the $\alpha 10$ integrin subunit.

Example 11

25 Immunohistochemical staining of $\alpha 10$ in fascia around tendon and skeletal muscle and in tendon structures in heart valves.

Materials and methods

Frozen sections of adult cartilage (trochlear
30 groove) obtained during surgery (provided by Anders Lindahl, Salgrenska Hospital, Gothenburg, Sweden and frozen sections from of 3 day old mouse limb were fixed and prepared for immunohistochemistry as earlier described (Camper et al, JBC, 273, 20383-20389 (1998)). Expression
35 of $\alpha 10$ integrin subunit was analysed using the polyclonal antibody against the cytoplasmic domain as a pri-

mary antibody (see Example 6) and a secondary antibody conjugated to peroxidase.

Results

As shown in figures 13 expression of $\alpha 10$ was found in the fascia surrounding tendon (a) and skeletal muscle (b) and in the tendon structures in the heart valves (c). This localisation suggest that $\alpha 10$ can bind to other matrix molecules in addition to the cartilage specific collagen type II. The localisation of the integrin $\alpha 10$ on the surface of tendons indicate that $\alpha 10$ can be involved in unwanted adhesion that often occurs between tendon/ligaments and the surrounding tissue after infection, injury or after surgery.

Example 12

mRNA expression of $\alpha 10$ integrin subunit in chondrocytes, endothelial cells and fibroblasts.

Material and methods

Isolation of mRNA, synthesis of cDNA and PCR amplification was done as earlier described (Camper et al, JBC, 273, 20383-20389 (1998)).

Results

Figure 14 shows PCR amplification of $\alpha 10$ cDNA from human articular chondrocytes (lanes A6 and B1), human umbilical vein endothelial cells (lane A2), human fibroblasts (lane A4) and rat tendon (Fig 14b, lane B2). Lanes 1, 3, and 5 in figure 14 A show amplified fragments corresponding to the integrin subunit $\alpha 2$ in endothelial cells, fibroblasts and chondrocytes, respectively. cDNA-primers corresponding to the $\alpha 10$ sequence positions nt 2919-2943 (forward) and nt 3554-3578 (reverse) (see Figure 6) were used to amplify $\alpha 10$ cDNA from the different cells. The figure shows that $\alpha 10$ was amplified in all three cell types. Two fragments of $\alpha 10$ was amplified which represent the intact form of $\alpha 10$ (larger fragment) and a splice variant (smaller fragment). The larger frag-

ment was dominating in chondrocytes while the smaller fragment was more pronounced in tendon (B2).

Example 13

5 Construction of $\alpha 10$ mammalian expression vector:

The full length protein coding sequence of $\alpha 10$ (combined from 3 clones, see figure 6) was inserted into the mammalian expression vector, pcDNA3.1/Zeo (Invitrogen). The vector contains SV40 promoter and Zeosin selection
10 sequence. The $\alpha 10$ containing expression vector was transfected into cells that express the $\beta 1$ -integrin subunit but lack expression of the $\alpha 10$ subunit. Expression of the $\alpha 10$ integrin subunit on the cell surface can be analysed by immunoprecipitation and/or flow cytometry using anti-
15 bodies specific for $\alpha 10$. The ligand binding capacity and the function of the inserted $\alpha 10$ integrin subunit can be demonstrated in cell adhesion experiment and in signalling experiments.

20 Example 14

Construction of mammalian expression vector containing a splice variant of $\alpha 10$.

The full length protein coding sequence of the splice variant of $\alpha 10$ (nt 2942-nt3055 deleted) was
25 inserted into the mammalian expression vector pcDNA3 (see Example 13). Expression and function of the splice variant can be analysed as described in example 13 and compared with the intact $\alpha 10$ integrin subunit.

30 Example 15

Partial isolation and characterisation of the $\alpha 10$ integrin genomic DNA

Material and methods

Human $\alpha 10$ cDNA, isolated from the racel-containing
35 plasmid using the restriction enzymes *Bam*HI (GIBCO BRL) and *Nco*I (Boehringer Mannheim), was 32 P-labelled and used as a probe for screening of a mouse 129 cosmid library

(provided by Reinhard Fässler, Lund University). Positive clones were isolated and subcloned. Selected plasmids were purified and sequenced as described earlier (Camper et al, JBC, 273, 20383-20389 (1998)) using T3, T7 and
5 internal specific primers. Primers corresponding to mouse genomic DNA were then constructed and used in PCR to amplify and identify the genomic sequence of $\alpha 10$ from the cosmid clones.

Results

10 Figure 15 shows 7958 nt of the $\alpha 10$ gene. This partial genomic DNA sequence of $\alpha 10$ integrin contains 8 exons, and a Kozak sequence. The mouse genomic $\alpha 10$ sequence was used to generate a targeting vector for knockout experiments.

15

Example 16

Upregulation of $\alpha 10$ integrin subunit in chondrocytes cultured in alginate beads

Material and methods

20 Human chondrocytes cultured in monolayer for 2 weeks were detached with trypsin-EDTA and introduced into alginate beads. Chondrocytes cultured in alginate are known to preserve their phenotype while chondrocytes cultured in monolayer are dedifferentiated. After 11 days chondro-
25 cytes cultured either in alginate or on monolayer were isolated and surface labelled with ^{125}I . The $\alpha 10$ integrin subunit was then immunoprecipitated with polyclonal antibodies recognising the cytoplasmic domain of $\alpha 10$ (see Example 6 and Camper et al, JBC, 273, 20383-20389
30 (1998)).

Results

As shown in figure 16 chondrocytes cultured in alginate beads (lanes 3 and 4) upregulated their protein expression of $\alpha 10\beta 1$. This was in contrast to chondrocytes
35 cultured in monolayer (lanes 1 and 2) which had a very low expression of $\alpha 10\beta 1$. Immunoprecipitation with ab control antibody is shown in lanes 1 and 3. It is known that

chondrocytes preserve their cartilage specific matrixproduction in alginate cultures but not in monolayer culture which point to that alginate preserve the phenotype of chondrocytes. These results support that $\alpha 10$ integrin subunit can be used as a marker for differentiated chondrocytes.

Example 17

Immunoprecipitation of the $\alpha 10$ integrin subunit from human smooth muscle cells.

Material and methods

Human smooth muscle cells were isolated from human aorta. After one week in culture the cells were ^{125}I -labelled, lysed and immunoprecipitated with antibodies against the integrin subunit $\beta 1$ (lane 1), $\alpha 1$ (lane 2), $\alpha 2$ (lane 3), $\alpha 10$ (lane 4), $\alpha 3$ (lane 5), control (lane 6) (Figure 17). The experiment was done as described in Example 7.

Results

The $\alpha 10$ antibody precipitated two bands from the smooth muscle cells corresponding to the $\alpha 10$ and the $\beta 1$ integrin subunit (Fig. 17).

Example 18

Construction of bacterial expression vector containing sequence for $\alpha 10$ splice region.

A plasmid for intracellular expression in *E. coli* of the alternatively spliced region (amino acid pos. 952-986, SEQ. ID 1) was constructed as described. The alternatively spliced region were back-translated using the *E. coli* high frequency codon table, creating a cDNA sequence of 96% identity with the original sequence (SEQ. ID 1 nucleotide pos 2940-3044). Using sequence overlap extension (Horton et al., Biotechniques 8:528, 1990) primer $\alpha 10\text{pfor}$ (tab. I) and $\alpha 10\text{prev}$ (tab. I) was used to generate a double stranded fragment encoding the $\alpha 10$ amino acid sequence. This fragment was used as a PCR

template with primers $\alpha 10$ pfor2 (tab. I) and $\alpha 10$ prev2 (tab. I) in order to generate restriction enzyme site for sub-cloning in a pET vector containing the Z-domain of staphylococcal protein A, creating a fusion of the $\alpha 10$ spliced region with the amino terminal of the Z-domain with trombin cleavage site residing in-between. The fragment generated in the second PCR reaction is shown (SEQ ID No. 3) also indicating the unique restriction enzymes used for sub-cloning in the expression vector.

10

Table I

$\alpha 10$ pfor	5'- G TTCAGAACCTGGGTTGCTACGTTGTTCCGGTCTGATCATCTCCGC TCTGCTGCCGGCTGT-3'
$\alpha 10$ pfor2	5'-GGGGCATATGGTTCAGAACCTGGGTTGCTACGTTG-3'
$\alpha 10$ prev	5'- GATAACCTGGGACAAGCTTAGGAAGTAGTTACCACCGTGAGCAACAG CCGGCAGCAGAGCGGA-3'
$\alpha 10$ prev2	5'- GGGGGGATCCGCGCGGCACCAGGCCGCTGATAACCTGGGACAAGCTT AGGAAGT-3'

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) NUMBER OF SEQUENCES: 2

(2) INFORMATION FOR SEQ ID NO. 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3884 base pairs
(B) TYPE: nucleic acid and amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (E) ORGANISM: human
(F) CELLTYPE: chondrocyte

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 1:

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(2) INFORMATION FOR SEQ ID NO. 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3779 base pairs
- (B) TYPE: nucleic acid and amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- (E)

(i) MOLECULAR TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: human
- (B) CELLTYPE: chondrocyte

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 2:

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TACCAACTGGGAAATTCATCTCATCCTGCTGTGAATATGCACCTGGGGATGTCTCTGTTA
301 -----+-----+-----+-----+-----+-----+-----+ 360
ATGGTTGACCCTTTAAGTAGAGTAGGACGACACTTATACGTGGACCCCTACAGAGACAAT

a   Y Q L G N S S H P A V N M H L G M S L L -

GAGACAGATGGTGATGGGGGATTCATGGCCTGTGCCCCTCTCTGGTCTCGTGCTTGTGGC
361 -----+-----+-----+-----+-----+-----+-----+ 420
CTCTGTCTACCACTACCCCTAAGTACCGGACACGGGGAGAGACCAGAGCACGAACCCG

a   E T D G D G G F M A C A P L W S R A C G -

AGCTCTGTCTTCAGTTCTGGGATATGTGCCCCTGTGGATGCTTCATTCCAGCCTCAGGGA
421 -----+-----+-----+-----+-----+-----+-----+ 480
TCGAGACAGAAGTCAAGACCCTATACACGGGCACACCTACGAAGTAAGGTCCGAGTCCCT
```

44

a S S V F S S G I C A R V D A S F Q P Q G -
AGCCTGGCACCCTGCCCCACGCTGCCCCAACATACATGGATGTTGTCTTGGAT
481 -----+-----+-----+-----+-----+ 540
TCGGACCGTGGGTGACGGGTGCGACGGGTGTATGTACCTACAACAGTAACAGAACCTA

a S L A P T A Q R C P T Y M D V V I V L D -
GGCTCCAACAGCATCTACCCCTGGTCTGAAGTTCAGACCTTCCTACGAAGACTGGTAGGG
541 -----+-----+-----+-----+-----+ 600
CCGAGGTGTGCTAGATGGGGACCAGACTTCAAGTCTGGAAGGATGCTTCTGACCATCCC

a G S N S I Y P W S E V Q T F L R R L V G -
AACTGTTTATTGACCCAGAACAGATACAGGTGGGACTGGTACAGTATGGGGAGAGCCCT
601 -----+-----+-----+-----+-----+ 660
TTTGACAAATAACTGGGTCTTGTCTATGTCCACCCTGACCATGTATACCCCTCTCGGGA

a K L F I D P E Q I Q V G L V Q Y G E S P -
GTACATGAGTGGTCCCTGGGAGATTTCGAACGAAGGAAGAAGTGGTGAGAGCAGCAAAG
661 -----+-----+-----+-----+-----+ 720
CATGTACTCACCAGGGACCCTCTAAAGGCTTGCTTCTTCTTACCCTCTCGTCGTTTC

a V H E W S L G D F R T K E E V V R A A K -
AACCTCAGTCGGCGGGAGGGACGAGAAACAAAGACTGCCAAGCAATAATGGTGGCCTGC
721 -----+-----+-----+-----+-----+ 780
TTGGAGTCAGCCGCCCTCCCTGCTCTTGTCTTGACGGGTTCGTTATTACCACCGGACG

a N L S R R E G R E T K T A Q A I M V A C -
ACAGAAGGGTTCAGTCAGTCCCATGGGGGCCGACCCGAGGCTGCCAGGCTACTGGTGGTT
781 -----+-----+-----+-----+-----+ 840
TGTCTTCCCAAGTCAGTCAGGTACCCCGGCTGGGCTCCGACGGTCCGATGACCACCAA

a T E G F S Q S H G G R P E A A R L L V V -
GTCAGTATGGAGAGTCCCATGATGGAGAGGAGCTTCCTGCAGCACTAAAGGCCTGTGAG
841 -----+-----+-----+-----+-----+ 900
CAGTGACTACCTCTCAGGGTACTACCTCTCCTCGAAGGACGTCGTGATTTCGGGACACTC

a V T D G E S H D G E E L P A A L K A C E -
GCTGGAAGAGTGACACGCTATGGGATTGCAGTCCTTGGTCACTACCTCCGGCGGCAGCGA
901 -----+-----+-----+-----+-----+ 960
CGACCTTCTCACTGTGCGATACCCTAACGTCAGGAACCAAGTATGGAGGCCCGCTCGCT

a A G R V T R Y G I A V L G H Y L R R Q R -
GATCCCAGCTCTTTCCTGAGAGAAATTAGAACTATTGCCAGTGATCCAGATGAGCGATT
961 -----+-----+-----+-----+-----+ 1020
CTAGGGTCGAGAAAGGACTCTCTTTAATCTTGATAACGGTCACTAGGTCTACTCGCTAAG

a D P S S F L R E I R T I A S D P D E R F -
TTCTTCAATGTCACAGATGAGGCTGCTCTGACTGACATTGTGGATGCACTAGGAGATCGG
1021 -----+-----+-----+-----+-----+ 1080
AAGAAGTTACAGTGTCTACTCCGACGAGACTGACTGTAACACCTACGTGATCCTCTAGCC

a F F N V T D E A A L T D I V D A L G D R -
ATTTTTGGCCTTGAAGGGTCCCATGCAGAAAACGAAAGCTCCTTGGGCTGGAAATGTCT
1081 -----+-----+-----+-----+-----+ 1140
TAAAAACCGAACTTCCAGGGTACGTCTTTTGCTTTCGAGGAAACCCGACCTTTACAGA

45

a I F G L E G S H A E N E S S F G L E M S -
CAGATTGGTTTCTCCACTCATCGGCTAAAGGATGGGATTCTTTTTGGGATGGTGGGGGCC
1141 -----+-----+-----+-----+-----+-----+ 1200
GTCTAACCAAAGAGGTGAGTAGCCGATTTCCTACCCTAAGAAAAACCTACCACCCCCGG

a Q I G F S T H R L K D G I L F G M V G A -
TATGACTGGGGAGGCTCTGTGCTATGGCTTGAAGGAGGCCACCGCCTTTTCCCCCACGA
1201 -----+-----+-----+-----+-----+-----+ 1260
ATACTGACCCCTCCGAGACACGATACCGAACTTCCTCCGGTGGCGGAAAAGGGGGGTGCT

a Y D W G G S V L W L E G G H R L F P P R -
ATGGCACTGGAAGACGAGTTCCTCCCTGCACTGCAGAACCATGCAGCCTACCTGGGTTAC
1261 -----+-----+-----+-----+-----+-----+ 1320
TACCGTGACCTTCTGCTCAAGGGGGACGTGACGTCTTGGTACGTCGGATGGACCCAATG

a M A L E D E F P P A L Q N H A A Y L G Y -
TCTGTTTCTTCCATGCTTTTGC GG GTGGACGCCGCTGTTTCTCTCTGGGGCTCCTCGA
1321 -----+-----+-----+-----+-----+-----+ 1380
AGACAAAGAAGGTACGAAAACGCCCCACCTGCGGCGGACAAAGAGAGACCCCGAGGAGCT

a S V S S M L L R G G R R L F L S G A P R -
TTTAGACATCGAGGAAAAGTCATCGCCTTCCAGCTTAAGAAAGATGGGGCTGTGAGGGTT
1381 -----+-----+-----+-----+-----+-----+ 1440
AAATCTGTAGCTCCTTTTCAGTAGCGGAAGGTCAATTCTTTCTACCCCGACACTCCCAA

a F R H R G K V I A F Q L K K D G A V R V -
GCCCAGAGCCTCCAGGGGGAGCAGATTGGTTCATACTTTGGCAGTGAGCTCTGCCATTG
1441 -----+-----+-----+-----+-----+-----+ 1500
CGGGTCTCGGAGGTCCCCCTCGTCTAACCAAGTATGAAACCGTCACTCGAGACGGGTAAC

a A Q S L Q G E Q I G S Y F G S E L C P L -
GATACAGATAGGGATGGAACAACCTGATGTCTTACTTGTGGCTGCCCCATGTTCTTGGGA
1501 -----+-----+-----+-----+-----+-----+ 1560
CTATGTCTATCCCTACCTTGTGACTACAGAATGAACACCGACGGGGGTACAAGGACCTT

a D T D R D G T T D V L L V A A P M F L G -
CCCCAGAACAAGGAAACAGGACGTGTTTATGTGTATCTGGTAGGCCAGCAGTCTTGCTG
1561 -----+-----+-----+-----+-----+-----+ 1620
GGGGTCTTGTTCCTTTGTCCTGCACAAATACACATAGACCATCCGGTCTGTCAGGAACGAC

a P Q N K E T G R V Y V Y L V G Q Q S L L -
ACCCTCCAAGGAACACTTCAGCCAGAACCCCCCAGGATGCTCGGTTTGGCTTTGCCATG
1621 -----+-----+-----+-----+-----+-----+ 1680
TGGGAGGTTCTTGTGAAGTCGGTCTTGGGGGGTCTACGAGCCAAACCGAAACGGTAC

a T L Q G T L Q P E P P Q D A R F G F A M -
GGAGCTCTTCTGATCTGAACCAAGATGGTTTTGCTGATGTGGCTGTGGGGGCGCCTCTG
1681 -----+-----+-----+-----+-----+-----+ 1740
CCTCGAGAAGGACTAGACTTGGTTCTACCAAACGACTACACCGACACCCCGCGGAGAC

a G A L P D L N Q D G F A D V A V G A P L -
GAAGATGGGCACCAGGGAGCACTGTACCTGTACCATGGAACCCAGAGTGGAGTCAGGCC
1741 -----+-----+-----+-----+-----+-----+ 1800
CTTCTACCCGTGGTCCCTCGTGACATGGACATGGTACCTTGGGTCTCACCTCAGTCCGGG

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a E D G H Q G A L Y L Y H G T Q S G V R P -
CATCCTGCCCAGAGGATTGCTGCTGCCTCCATGCCACATGCCCTCAGCTACTTTGGCCGA
1801 -----+-----+-----+-----+-----+ 1860
GTAGGACGGGTCTCCTAACGACGACGGAGGTACGGTGTACGGGAGTCGATGAAACCGGCT

a H P A Q R I A A A S M P H A L S Y F G R -
AGTGTGGATGGTCGGCTAGATCTGGATGGAGATGATCTGGTTCGATGTGGCTGTGGGTGCC
1861 -----+-----+-----+-----+-----+ 1920
TCACACCTACCAGCCGATCTAGACCTACCTCTACTAGACCAGCTACACCGACACCCACGG

a S V D G R L D L D G D D L V D V A V G A -
CAGGGGGCAGCCATCCTGCTCAGCTCCCGGCCATTGTCCATCTGACCCCATCACTGGAG
1921 -----+-----+-----+-----+-----+ 1980
GTCCCCCGTCGGTAGGACGAGTCGAGGGCCGGTAACAGGTAGACTGGGGTAGTGACCTC

a Q G A A I L L S S R P I V H L T P S L E -
GTGACCCACAGGCCATCAGTGTGGTTCAGAGGGACTGTAGGCGGCGAGGCCAAGAAGCA
1981 -----+-----+-----+-----+-----+ 2040
CACTGGGGTGTCCGGTAGTCACACCAAGTCTCCCTGACATCCGCCGCTCCGGTTCTTCGT

a V T P Q A I S V V Q R D C R R R G Q E A -
GTCTGTCTGACTGCAGCCCTTTGCTTCCAAGTGACCTCCCGTACTCCTGGTCGCTGGGAT
2041 -----+-----+-----+-----+-----+ 2100
CAGACAGACTGACGTCCGGAAACGAAGGTTCACTGGAGGGCATGAGGACCAGCGACCCTA

a V C L T A A L C F Q V T S R T P G R W D -
CACCAATTCTACATGAGGTTCCACCGCATCACTGGATGAATGGACTGCTGGGGCACGTGCA
2101 -----+-----+-----+-----+-----+ 2160
GTGGTTAAGATGTACTCCAAGTGGCGTAGTGACCTACTTACCTGACGACCCCGTGACGT

a H Q F Y M R F T A S L D E W T A G A R A -
GCATTTGATGGCTCTGGCCAGAGGTTGTCCCCTCGGAGGCTCCGGCTCAGTGTGGGGAAT
2161 -----+-----+-----+-----+-----+ 2220
CGTAAACTACCGAGACCGGTCTCCAACAGGGGAGCCTCCGAGGCCGAGTCACACCCCTTA

a A F D G S G Q R L S P R R L R L S V G N -
GTCATTGTGAGCAGCTACACTTCCATGTGCTGGATACATCAGATTACCTCCGGCCAGTG
2221 -----+-----+-----+-----+-----+ 2280
CAGTGAACACTCGTCGATGTGAAGGTACACGACCTATGTAGTCTAATGGAGGCCGGTCAC

a V T C E Q L H F H V L D T S D Y L R P V -
GCCTTGACTGTGACCTTTGCCTTGGACAATACTACAAAGCCAGGGCCTGTGCTGAATGAG
2281 -----+-----+-----+-----+-----+ 2340
CGGAAGTACACTGGAAACGGAACCTGTTATGATGTTTCGGTCCCGGACACGACTTACTC

a A L T V T F A L D N T T K P G P V L N E -
GGCTCACCACCTCTATACAAAAGCTGGTCCCCTTCTCAAAGGATTGTGGCCCTGACAAT
2341 -----+-----+-----+-----+-----+ 2400
CCGAGTGGGTGGAGATATGTTTTCGACCAGGGGAAGAGTTTCCTAACACCGGGACTGTTA

a G S P T S I Q K L V P F S K D C G P D N -
GAATGTGTACAGACCTGGTGCTTCAAGTGAATATGGACATCAGAGGCTCCAGGAAGGCC
2401 -----+-----+-----+-----+-----+ 2460
CTTACACAGTGTCTGGACCACGAAGTTCACCTATACCTGTAGTCTCCGAGGTCCTTCCGG

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a E C V T D L V L Q V N M D I R G S R K A -
CCATTTGTGGTTCGAGGTGGCCGGCGGAAAGTGCTGGTATCTACAACCTCTGGAGAACAGA
2461 -----+-----+-----+-----+-----+ 2520
GGTAAACACCAAGCTCCACCGGCCCTTTACGACCATAGATGTTGAGACCTCTTGTCT

a P F V V R G G R R K V L V S T T L E N R -
AAGGAAAATGCTTACAATACGAGCCTGAGTATCATCTTCTCTAGAAACCTCCACCTGGCC
2521 -----+-----+-----+-----+-----+ 2580
TTCCTTTTACGAATGTTATGCTCGGACTCATAGTAGAAGAGATCTTTGGAGGTGGACCGG

a K E N A Y N T S L S I I F S R N L H L A -
AGTCTCACTCCTCAGAGAGAGAGCCCAATAAAGGTGGAATGTGCCGCCCTTCTGCTCAT
2581 -----+-----+-----+-----+-----+ 2640
TCAGAGTGAGGAGTCTCTCTCTCGGGTTATTTCCACCTTACACGGCGGGGAAGACGAGTA

a S L T P Q R E S P I K V E C A A P S A H -
GCCCCGCTCTGCAGTGTGGGGCATCCTGTCTTCCAGACTGGAGCCAAGGTGACCTTTCTG
2641 -----+-----+-----+-----+-----+ 2700
CGGGCCGAGACGTCACACCCCGTAGGACAGAAGGTCTGACCTCGGTTCCACTGGAAGAC

a A R L C S V G H P V F Q T G A K V T F L -
CTAGAGTTTGAGTTTAGCTGCTCCTCTCTCCTGAGCCAGGTCTTTGGAAGCTGACTGCC
2701 -----+-----+-----+-----+-----+ 2760
GATCTCAAACCTCAAATCGACGAGGAGAGAGGACTCGGTCCAGAAACCCTTCGACTGACGG

a L E F E F S C S S L L S Q V F G K L T A -
AGCAGTGACAGCCTGGAGAGAAATGGCACCCCTTCAAGAAAACACAGCCCAGACCTCAGCC
2761 -----+-----+-----+-----+-----+ 2820
TCGTCACTGTGCGACCTCTCTTTACCGTGGGAAGTTCTTTTGTGTCGGGTCTGGAGTCGG

a S S D S L E R N G T L Q E N T A Q T S A -
TACATCCAATATGAGCCCCACCTCCTGTTCTCTAGTGAGTCTACCCTGCACCGCTATGAG
2821 -----+-----+-----+-----+-----+ 2880
ATGTAGGTTATACTCGGGGTGGAGGACAAGAGATCACTCAGATGGGACGTGGCGATACTC

a Y I Q Y E P H L L F S S E S T L H R Y E -
GTTCACCCATATGGGACCCTCCCAGTGGGTCCTGGCCCAGAATTCAAACCACTCTCAGG
2881 -----+-----+-----+-----+-----+ 2940
CAAGTGGGTATACCCTGGGAGGGTCACCCAGGACCGGCTTAAGTTTTGGTGAGAGTCC

a V H P Y G T L P V G P G P E F K T T L R -
ACTAACAATGCAAGCTGCATAGTGAGAACCTGACTGAACCCCCAGGCCACCTGTGCAT
2941 -----+-----+-----+-----+-----+ 3000
TGATTGTTACGTTTCGACGTATCACGTCTTGACTGACTTGGGGGTCCGGGTGGACACGTA

a T N N A S C I V Q N L T E P P G P P V H -
CCAGAGGAGCTTCAACACACAAACAGACTGAATGGGAGCAATACTCAGTGTGAGTGGTG
3001 -----+-----+-----+-----+-----+ 3060
GGTCTCCTCGAAGTTGTGTGTTGTCTGACTTACCCTCGTTATGAGTCACAGTCCACCAC

a P E E L Q H T N R L N G S N T Q C Q V V -
AGGTGCCACCTTGGGCAGCTGGCAAAGGGGACTGAGGTCTCTGTTGGACTATTGAGGCTG
3061 -----+-----+-----+-----+-----+ 3120
TCCACGGTGGAACCCGTGACCGTTCCCTGACTCCAGAGACAACCTGATAACTCCGAC

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a R C H L G Q L A K G T E V S V G L L R L -
GTT CACAATGAATTTTCCGAAGAGCCAAGTTCAAGTCCCTGACGGTGGTCAGCACCTTT
3121 -----+-----+-----+-----+-----+ 3180
CAAGTGTTACTTAAAAAGGCTTCTCGGTTCAAGTTCAGGGACTGCCACCAGTCGTGGAAA

a V H N E F F R R A K F K S L T V V S T F -
GAGCTGGGAACCGAAGAGGGCAGTGTCTACAGCTGACTGAAGCCTCCCGTTGGAGTGAG
3181 -----+-----+-----+-----+-----+ 3240
CTCGACCCTTGGCTTCTCCCGTCACAGGATGTCGACTGACTTCGGAGGGCAACCTCACTC

a E L G T E E G S V L Q L T E A S R W S E -
AGCCTCTTGGAGGTGGTTCAGACCCGGCCTATCCTCATCTCCCTGTGGATCCTCATAGGC
3241 -----+-----+-----+-----+-----+ 3300
TCGGAGAACCTCCACCAAGTCTGGGCCGGATAGGAGTAGAGGGACACCTAGGAGTATCCG

a S L L E V V Q T R P I L I S L W I L I G -
AGTGTCTTGGGAGGGTGTCTCTGCTTGTCTCTCTTGTCTTCTGCCTGTGGAAGCTTGGC
3301 -----+-----+-----+-----+-----+ 3360
TCACAGGACCCTCCCAACGAGGACGAACGAGAGGAACAGAAGACGGACACCTTCGAACCG

a S V L G G L L L L A L L V F C L W K L G -
TTCTTTGCCCATAGAAAAATCCCTGAGGAAGAAAAAGAGAAGAGAAGTTGGAGCAATGA
3361 -----+-----+-----+-----+-----+ 3420
AAGAAACGGGTATTCTTTTAGGGACTCCTTCTTTTCTCTTCTTCTTCAACCTCGTTACT

a F F A H K K I P E E E K R E E K L E Q
ATGTAGAATAAGGGTCTAGAAAGTCTCCCTGGCAGCTTTCTTCAAGAGACTTGCATAAA
3421 -----+-----+-----+-----+-----+ 3480
TACATCTTATTCCCAGATCTTTCAGGAGGGACCGTCGAAAGAAGTTCTCTGAACGTATTT

AGCAGAGGTTTGGGGGCTCAGATGGGACAAGAAGCCGCTCTGGACTATCTCCCCAGACC
3481 -----+-----+-----+-----+-----+ 3540
TCGTCTCAAACCCCGAGTCTACCCTGTTCTTCGGCGGAGACCTGATAGAGGGGTCTGG

AGCAGCCTGACTTGACTTTTGAGTCCTAGGGATGCTGCTGGCTAGAGATGAGGCTTTACC
3541 -----+-----+-----+-----+-----+ 3600
TCGTCTGACTGAACTGAAACTCAGGATCCCTACGACGACCGATCTCTACTCCGAAATGG

TCAGACAAGAAGAGCTGGCACCAAACTAGCCATGCTCCACCCCTCTGCTTCCCTCCTCC
3601 -----+-----+-----+-----+-----+ 3660
AGTCTGTTCTTCTCGACCGTGGTTTTGATCGGTACGAGGTGGGAGACGAAGGGAGGAGG

TCGTGATCCTGGTTCCATAGCCAACACTGGGGCTTTTGTGTTGGGGTCTTTTATCCCCAG
3661 -----+-----+-----+-----+-----+ 3720
AGCACTAGGACCAAGGTATCGGTTGTGACCCCGAAACAAACCCAGGAAAATAGGGGTC

GAATCAATAATTTTTTGCCTAGGAAAAAAAAGCGGCCGCGAATTCGATATCAAGCT
3721 -----+-----+-----+-----+-----+ 3779
CTTAGTTATTAAAAAACGGATCCTTTTTTTTTTCGCCGGCGCTTAAGCTATAGTTCGA

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(2) INFORMATION FOR SEQ ID NO. 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 143 base pairs
- (B) TYPE: nucleic acid and amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(iii) MOLECULAR TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: human
- (B) CELLTYPE: chondrocyte

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 3:

```

      NdeI
      |
GGGGCATATGGTTCAGAACCTGGGTTGCTACGTTGTTTCCGGTCTGATCATCTCCGCTCT
1  -----+-----+-----+-----+-----+-----+ 60
CCCCGTATACCAAGTCTTGGACCCAACGATGCAACAAAGGCCAGACTAGTAGAGGCGAGA

b      G H M V Q N L G C Y V V S G L I I S A L -

      GCTGCCGGCTGTTGCTCACGGTGGTAACTACTTCCTAAGCTTGTCCCAGGTATCAGCGG
61 -----+-----+-----+-----+-----+-----+ 120
CGACGGCCGACAACGAGTGCCACCATTGATGAAGGATTGGAACAGGGTCCAATAGTCGCC

b      L P A V A H G G N Y F L S L S Q V I S G -

      BamHI
      |
CCTGGTGCCGCGCGGATCCCCC
121 -----+-----+----- 143
GGACCACGGCGCGCCTAGGGGGG

b      L V P R G S P -
```


CLAIMS

1. A recombinant or isolated integrin subunit $\alpha 10$
5 comprising the amino acid sequence shown in SEQ ID No. 1
or SEQ ID No. 2, or homologues or fragments thereof hav-
ing similar biological activity.
2. A process of producing a recombinant integrin
subunit $\alpha 10$ comprising the amino acid sequence shown in
10 SEQ ID No. 1 or SEQ ID No. 2, or homologues or fragments
thereof having similar biological activity, which process
comprises the steps of
 - a) isolating a polynucleotide comprising a nucleo-
tide sequence coding for an integrin subunit $\alpha 10$, or
15 homologues or fragments thereof having similar biological
activity,
 - b) constructing an expression vector comprising the
isolated polynucleotide,
 - c) transforming a host cell with said expression
20 vector,
 - d) culturing said transformed host cell in a culture
medium under conditions suitable for expression of inte-
grin subunit $\alpha 10$, or homologues or fragments thereof hav-
ing similar biological activity, in said transformed host
25 cell, and, optionally,
 - e) isolating the integrin subunit $\alpha 10$, or homologues
or fragments thereof having similar biological activity,
from said transformed host cell or said culture medium.
3. A process of providing an integrin subunit $\alpha 10$,
30 or homologues or fragments thereof having similar biolo-
gical activity, whereby said subunit is isolated from a
cell in which it is naturally present.
4. An isolated polynucleotide comprising a nucleo-
tide coding for an integrin subunit $\alpha 10$, or for homolo-
35 gues or fragments thereof, which polynucleotide comprises
the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID
No. 2 or suitable parts thereof.

5. An isolated polynucleotide or oligonucleotide which hybridises to a DNA or RNA encoding an integrin subunit $\alpha 10$ or homologues or fragments thereof, wherein said polynucleotide or oligonucleotide fails to
5 hybridise to a DNA or RNA encoding an integrin subunit $\alpha 1$.

6. A vector comprising a polynucleotide or oligonucleotide coding for an integrin subunit $\alpha 10$, or homologues or fragments thereof, which polynucleotide or oligonucleotide comprises the nucleotide sequence shown in
10 SEQ ID No. 1 or SEQ ID No. 2 or parts thereof.

7. A vector comprising a polynucleotide or oligonucleotide which hybridises to a DNA or RNA encoding an integrin subunit $\alpha 10$ or homologues or fragments thereof,
15 wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit $\alpha 1$.

8. A cell containing the vector as defined in any one of claims 6 and 7.

20 9. A cell generated by the process in claim 2, in which a polynucleotide or oligonucleotide coding for an integrin subunit $\alpha 10$, or homologues or fragments thereof, which polynucleotide or oligonucleotide comprises the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2
25 or parts thereof has been stably integrated in the cell genome.

10. Binding entities having the capability of binding specifically to integrin subunit $\alpha 10$ comprising the amino acid sequence of SEQ ID No. 1 or SEQ ID No. 2, or
30 to homologues or fragments thereof.

11. Binding entities according to claim 10, which are chosen from the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, polyclonal and monoclonal antibodies, and fragments
35 thereof.

12. A recombinant or isolated integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , in which the

subunit $\alpha 10$ comprises the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, and homologues and fragments thereof having similar biological activity.

13. A recombinant or isolated integrin heterodimer
5 according to claim 12, wherein the subunit β is $\beta 1$.

14. A process of producing a recombinant integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , in which the subunit $\alpha 10$ comprises the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, and homologues and
10 fragments thereof, which process comprises the steps of

a) isolating one polynucleotide comprising a nucleotide sequence coding for a subunit $\alpha 10$ of an integrin heterodimer and, optionally, another polynucleotide comprising a nucleotide sequence coding for a subunit β of
15 an integrin heterodimer, or polynucleotides or oligonucleotides coding for homologues or fragments thereof having similar biological activity,

b) constructing an expression vector comprising said isolated polynucleotide coding for said subunit $\alpha 10$
20 optionally in combination with an expression vector comprising said isolated nucleotide coding for said subunit β ,

c) transforming a host cell with said expression vector or vectors,

25 d) culturing said transformed host cell in a culture medium under conditions suitable for expression of an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or homologues or fragments thereof having similar biological activity, in said transformed host cell, and,
30 optionally,

e) isolating the integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or homologues or fragments thereof having similar biological activity, or the $\alpha 10$ subunit thereof from said transformed host cell or said
35 culture medium.

15. A process of providing an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or homologues

or fragments thereof having similar biological activity, whereby said integrin heterodimer is isolated from a cell in which it is naturally present.

16. A cell containing a first vector, said first
5 vector comprising a polynucleotide or oligonucleotide coding for a subunit $\alpha 10$ of an integrin heterodimer, or for homologues or parts thereof having similar biological activity, which polynucleotide or oligonucleotide comprises the nucleotide sequence shown in SEQ ID No. 1 or
10 SEQ ID No. 2 or parts thereof, and a second vector, said second vector comprising a polynucleotide or oligonucleotide coding for a subunit β of an integrin heterodimer, or for homologues or fragments thereof.

17. Binding entities having the capability of binding specifically to the integrin heterodimer comprising
15 a subunit $\alpha 10$ and a subunit β , or to homologues or fragments thereof, or a subunit $\alpha 10$ thereof, having similar biological activity.

18. Binding entities according to claim 17, wherein
20 the subunit β is $\beta 1$.

19. Binding entities according to claim 17 or 18, which are chosen among the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, and fragments thereof.

20. A fragment of the integrin subunit $\alpha 10$, which
25 fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

21. A fragment according to claim 20, which is a
30 peptide comprising the amino acid sequence
KLGFFAHKKIPEEEKREEKLEQ.

22. A fragment according to claim 20, which comprises the amino acid sequence from about amino acid
No. 952 to about amino acid no. 986 of SEQ ID No. 1.

23. A fragment according to claim 20, which is a
35 peptide comprising the amino acid sequence from about

amino acid No. 140 to about amino acid no. 337 of
SEQ ID No. 1.

24. A method of producing a fragment of the integrin
subunit $\alpha 10$ as defined in any one of claims 20-23, which
5 method comprises a sequential addition of amino acids
containing protective groups.

25. A polynucleotide or oligonucleotide coding for
a fragment of the integrin subunit $\alpha 10$ as defined in any
one of claims 20-23.

10 26. Binding entities having the capability of bind-
ing specifically to a fragment of the human integrin sub-
unit $\alpha 10$ as defined in any one of claims 20-23.

27. Binding entities according to claim 26, which
are chosen from the group comprising proteins, peptides,
15 carbohydrates, lipids, natural integrin binding ligands,
and fragments thereof.

28. A process of using an integrin subunit $\alpha 10$ com-
prising the amino acid sequence shown in SEQ ID No. 1 or
SEQ ID No. 2, or an integrin heterodimer comprising said
20 subunit $\alpha 10$ and a subunit β , or a homologue or fragment
of said integrin or subunit having similar biological
activity, as a marker or target molecule of cells or tis-
sues expressing said integrin subunit $\alpha 10$, which cells or
tissues are of animal including human origin.

25 29. A process according to claim 28, whereby said
fragment is a peptide chosen from the group comprising
peptides of the cytoplasmic domain, the I-domain and the
spliced domain.

30 30. A process according to claim 29, whereby said
fragment is a peptide comprising the amino acid sequence
KLGFFAHKKIPEEEKREEKLEQ.

31. A process according to claim 29, whereby said
fragment comprises the amino acid sequence from about
amino acid no. 952 to about amino acid no. 986 of
35 SEQ ID No. 1.

32. A process according to claim 29, whereby said
fragment comprises the amino acid sequence from about

amino acid no. 140 to about amino acid no. 337 of
SEQ ID No. 1.

33. A process according to claim 28, whereby the
subunit β is $\beta 1$.

5 34. A process according to claim 28, whereby said
cells are chosen from the group comprising chondrocytes,
smooth muscle cells, endothelial cells, osteoblasts and
fibroblasts.

10 35. A process according to any one of claims 28-34,
which process is used during pathological conditions
involving said subunit $\alpha 10$.

36. A process according to claim 35, which patho-
logical conditions comprise damage of cartilage.

15 37. A process according to claim 36, which patho-
logical conditions comprise trauma, rheumatoid arthritis
and osteoarthritis.

38. A process according to any one of claims 28-34,
which is a process for detecting the formation of car-
tilage during embryonal development.

20 39. A process according to any one of claims 28-34,
which is a process for detecting physiological or thera-
peutic reparation of cartilage.

40. A process according to any one of claims 28-34,
which is a process for selection and analysis, or for
25 sorting, isolating or purification of chondrocytes.

41. A process according to any one of claims 28-34,
which is a process for detecting regeneration of carti-
lage or chondrocytes during transplantation of cartilage
or chondrocytes.

30 42. A process according to any one of claims 28-34,
which is a process for in vitro studies of differentia-
tion of chondrocytes.

43. A process of using binding entities having the
capability of binding specifically to an integrin subunit
35 $\alpha 10$ comprising the amino acid sequence shown in SEQ ID
No. 1 or SEQ ID No. 2, or an integrin heterodimer com-
prising said subunit $\alpha 10$ and a subunit β , or to homo-

logues or fragments thereof having similar biological activity, as markers or target molecules of cells or tissues expressing said integrin subunit $\alpha 10$, which cells or tissues are of animal including human origin.

5 44. A process according to claim 43, whereby said fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

10 45. A process according to claim 43, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

15 46. A process according to claim 43, whereby said fragment comprises the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

20 47. A process according to claim 43, whereby said fragment comprises the amino acid sequence from about amino acid no. 140 to about amino acid No. 337 of SEQ ID No. 1.

20 48. A process according to claim 43, whereby the subunit β is $\beta 1$.

25 49. A process according to any one of claims 43-48, which is a process for detecting the presence of an integrin subunit $\alpha 10$ comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or of an integrin heterodimer comprising said subunit $\alpha 10$ and a subunit β , or of homologues or fragments thereof having similar biological activity.

30 50. A process according to any one of claims 43-48, which process is a process for determining the differentiation-state of cells during embryonic development, angiogenesis, or development of cancer.

35 51. A process for detecting the presence of an integrin subunit $\alpha 10$, or of a homologue or fragment of said integrin subunit having similar biological activity, on cells, whereby a polynucleotide or oligonucleotide chosen from the group comprising a polynucleotide or oligo-

nucleotide shown in SEQ ID No. 1 is used as a marker under hybridisation conditions wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit $\alpha 1$.

5 52. A process according to claim 51, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

10 53. A process according to claim 51, whereby said fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

15 54. A process according to claim 53, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

20 55. A process according to claim 53, whereby said fragment comprises the amino acid sequence from about amino acid No. 952 to about amino acid no. 986 of SEQ ID No. 1.

20 56. A process according to claim 53, whereby said fragment comprises the amino acid sequence from about amino acid No. 140 to about amino acid No. 337 of SEQ ID No. 1.

25 57. A process according to any one of claims 43-48, which is a process for determining the differentiation-state of cells during development, in pathological conditions, in tissue regeneration or in therapeutic and physiological reparation of cartilage.

30 58. A process according to claim 57, wherein the pathological conditions are any pathological conditions involving the integrin subunit $\alpha 10$.

35 59. A process according to claim 58, whereby said pathological conditions are rheumatoid arthritis, osteoarthritis or cancer.

35 60. A process according to claim 57, whereby said cells are chosen from the group comprising chondrocytes,

smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

61. A process for determining the differentiation-state of cells during development, in pathological conditions, in tissue regeneration and in therapeutic and physiological repair of cartilage, whereby a polynucleotide or oligonucleotide chosen from the nucleotide sequence shown in SEQ ID No. 1 is used as a marker under hybridisation conditions wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit $\alpha 1$.

62. A process according to claim 61, whereby said polynucleotide or oligonucleotide is a polynucleotide or oligonucleotide coding for a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

63. A process according to claim 62, whereby said polynucleotide or oligonucleotide is a polynucleotide or oligonucleotide coding for a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

64. A process according to claim 62, whereby said peptide comprises the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

65. A process according to claim 62, whereby said peptide comprises the amino acid sequence from about amino acid no. 140 to about amino acid no. 337 of SEQ ID No. 1.

66. A process according to claim 61, whereby said pathological conditions are any pathological conditions involving the integrin subunit $\alpha 10$.

67. A process according to claim 66, whereby said pathological conditions are rheumatoid arthritis, osteoarthritis or cancer.

68. A process according to claim 66, whereby said pathological conditions are atherosclerosis or inflammation.

69. A process according to any one of claims 61-68, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

5 70. A pharmaceutical composition comprising as an active ingredient a pharmaceutical agent or an antibody which is capable of using an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or
10 subunit $\alpha 10$ having similar biological activity, as a target molecule.

71. A pharmaceutical composition according to claim 70, for use in stimulating, inhibiting or blocking the formation of cartilage, bone or blood vessels.

15 72. A pharmaceutical composition according to claim 70, for use in preventing adhesion between tendon/ligaments and the surrounding tissue after infection, inflammation and after surgical intervention where adhesion impairs the function of the tissue.

20 73. A vaccine comprising as an active ingredient an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or subunit $\alpha 10$, or DNA or RNA coding for said integrin subunit $\alpha 10$.

25 74. Use of the integrin subunit $\alpha 10$ as a marker or target in transplantation of cartilage or chondrocytes.

75. A method of using binding entities having the capability of binding specifically to an integrin subunit $\alpha 10$ comprising the amino acid sequence shown in SEQ ID
30 No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit $\alpha 10$ and a subunit β , or to homologues or fragments thereof having similar biological activity, for promoting adhesion of chondrocytes and/or osteoblasts to surfaces of implants to stimulate osseo-
35 integration.

76. Use of an integrin heterodimer comprising an integrin subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$

thereof, or a homologue or fragment of said integrin or subunit $\alpha 10$ having similar biological activity, as a target for anti-adhesive drugs or molecules in tendon, ligament, skeletal muscle or other tissues where adhesion
5 impairs the function of the tissue.

77. A method of stimulating, inhibiting or blocking the formation of cartilage or bone, comprising administration to a subject a suitable amount of a pharmaceutical agent or an antibody which is capable of using an
10 integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or subunit $\alpha 10$ having similar biological activity, as a target molecule.

78. A method of preventing adhesion between tendon/
15 ligaments and the surrounding tissue after infection, inflammation and after surgical intervention where adhesion impairs the function of the tissue, comprising administration to a subject a suitable amount of a pharmaceutical agent or an antibody which is capable of using a
20 integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or subunit $\alpha 10$ having similar biological activity, as a target molecule.

79. A method of stimulating extracellular matrix
25 synthesis and repair by activation or blockage of an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or of the subunit $\alpha 10$ thereof, or of a homologue or fragment of said integrin or subunit $\alpha 10$ having similar biological activity.

80. A method of in vitro detecting the presence of
30 integrin binding entities, comprising interaction of an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or subunit, with a sample,
35 thereby causing said integrin, subunit $\alpha 10$, or homologue or fragment thereof having similar biological activity,

to modulate the binding to its natural ligand or other integrin binding proteins present in said sample.

81. A method of in vitro studying consequences of the interaction of a human heterodimer integrin comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or subunit, with an integrin binding entity and thereby initiate a cellular reaction.

82. A method according to claim 81, whereby the consequences of said interactions are measured as alterations in cellular functions.

83. A method of using DNA or RNA encoding an integrin subunit $\alpha 10$ or homologues or fragments thereof as a target molecule.

84. A method according to claim 83, whereby a polynucleotide or oligonucleotide hybridises to the DNA or RNA encoding an integrin subunit $\alpha 10$ or homologues or fragments thereof and whereby said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit $\alpha 1$.

85. A method of using a human heterodimer integrin comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or subunit, or a DNA or RNA encoding an integrin subunit $\alpha 10$ or homologues or fragments thereof, as a marker or target molecule during angiogenesis.

86. A pharmaceutical composition comprising as an active ingredient a pharmaceutical agent or an antibody which is capable of stimulating cell surface expression of an integrin heterodimer comprising a subunit $\alpha 10$ and a subunit β , or the subunit $\alpha 10$ thereof, or a homologue or fragment of said integrin or subunit $\alpha 10$ having similar biological activity.

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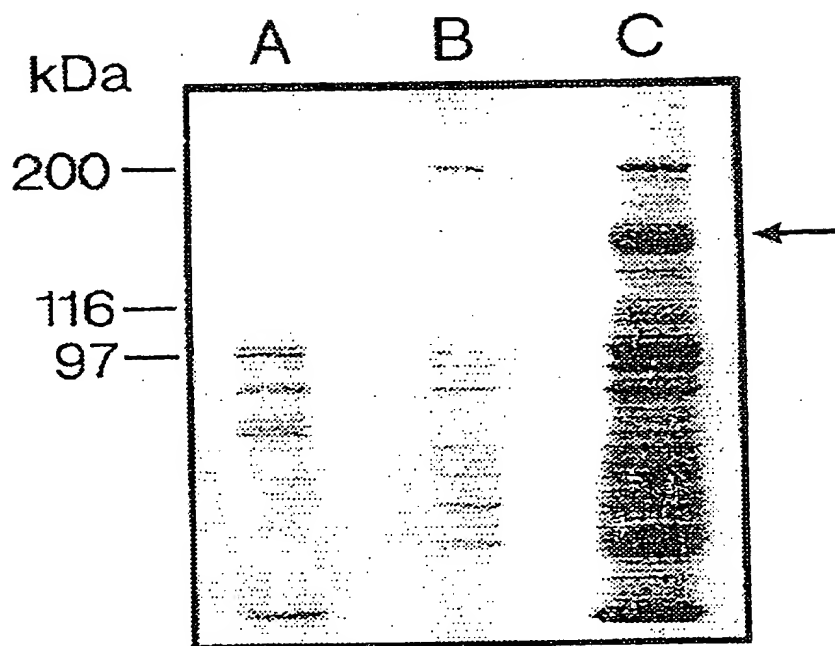


FIGURE 1

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Peptide	Amino acid sequence
1	DNTAQTSAYIQYEPHHSI
2	GPGHWDR
3	AAFDGSGQR
4	FAMGALPD
5	FTASLDEWTTAAR
6	VDASFRPQGXLAP

FIGURE 2

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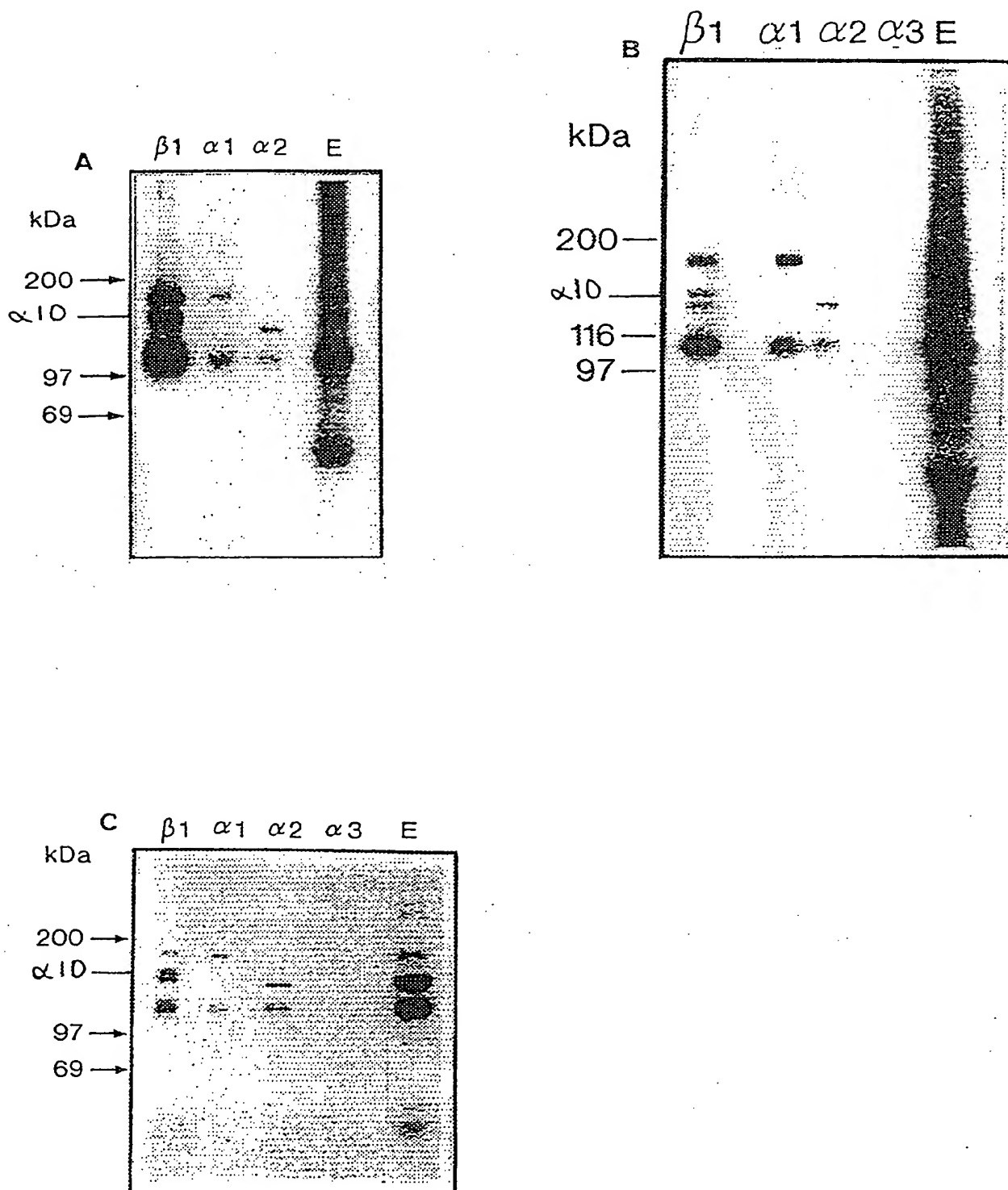


FIGURE 3

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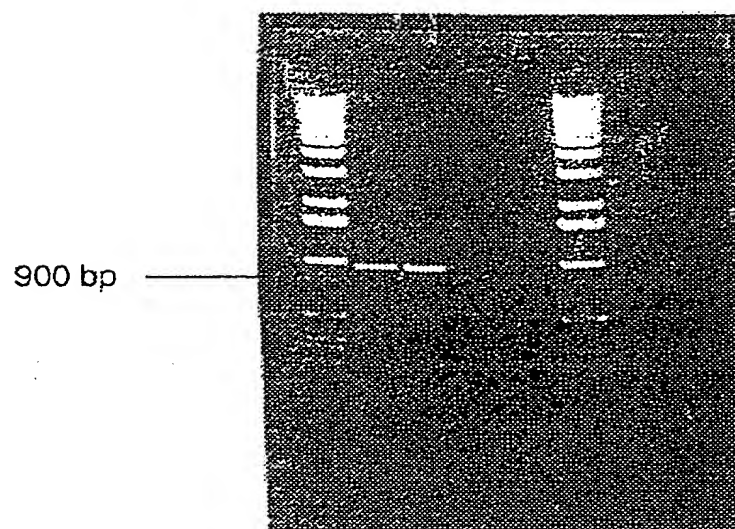


FIGURE 4

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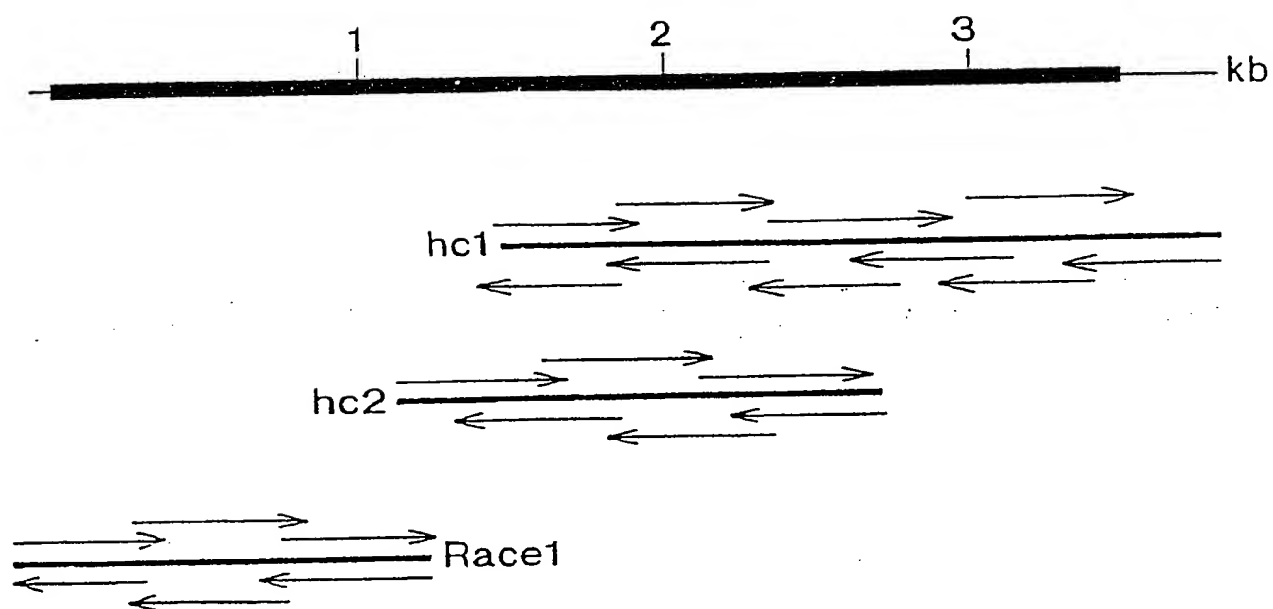


FIGURE 5

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CAGGT CAGAAACGATCAGCATGGAACTCCCTTCCTCACTCAGCTGTCTTCCCTCGTGTTCCTGACA	72	CATCTCGCCAGAGGATTCCTGCTGCTTCATGCCACATGCCCTCAGCTACTTGGCCGAAGTGTGGATGCT	1072
H E L P P V T H L F L P L V P L T	-6	H P A Q R I A A A S H P H A L S Y F G R S V D G	595
GGTCTCTGCTCCCTTTAACTCGATGAACATCACCCACCCCTATTCCAGCCGCCACAGAGCTGAATTT	144	CGCTAGATCTGGATGGACATGATCTGGTGGATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	1946
G L C S P F H L D E H N P R L F P G P P E A E F	19	R L D L D G D D L V D V A V G A Q G A A I L L S	619
GGATACAGTCTCTTACAACTGTGGGGTGGACAGCGATGATGCTGCTGGCGCCCTCGGATGGCCCT	216	TCCTGGCCATGTCTCATCTGACCCCATCTGAGGTGACCCACAGCCCATCACTGTGCTGACAGCGGAC	2016
G Y S V L O H V C G G G Q R W H L V C A P V D G P	43	S R P I V H L T P S L E V T P O A I S V V F O R D	643
TCAGCGACCGAGGGGGGAGTTTATCGCTGCCCTGTAGCGGGGCCACAAATGCCCATGTGCTCAAGGCG	288	TGTAGCGCGAGGCGCAAGAGCAGTCTGCTGATGCTGACCGCTTTGCTGCTCAAGTGACTGCTGCTGCT	2081
S G D R R G D V Y A C P V C G A H H A P C A X G	67	C P R R G Q E A V C L T A A L C F Q V T S R T	667
CACCTAGGTGACTACCAATGGGAATTCATCTCATCTCTGTAATATGACCTGGGAGTCTCTCTTTA	360	GGTGGTGGGATCACAATTCATAGAGGTTCACCGCATCACTGGAATGAATGGAGTCTGGGCGACCTGCA	2104
H L G D Y Q L G N S S H P A V M H H L G R S L L	91	G R M D H O P Y M R F T A S L D E M T A G A R A	691
GAGACAGATGGTATGGGGATTCATGCTGTGCTCTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	432	GCATTTGATGGCTCTGGCCAGAGTGTGCTGCTGGAGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	2232
E T D G C D G G G F N A C A P L M S R A C G S S V F	115	A F D G S G Q R L S P R R L R L S V G H V T C E	715
AGTCTGGGATATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	504	CAGTACAGTTCATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	2304
S S T T C G G A T A T G C T G C T G C T G C T G C T G C T G C T G C T G C T G C T G C T G C T	139	Q L H T V L D T S D Y L R P V L D M T A G A R A	739
TGCCCAACATACATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	576	GACAACTACAAAGCCAGGCGCTGCTGCTGAATGAGGCGTCAACCCCTCTATACAAAGCTGGTCTCTTC	2376
C P T T Y M D V V I V L D G S N S I Y P W S E V Q	163	D N T T K P G P V L N E G S P T S I C K L V P F	763
ACCTTCTACGAGACGTTAGGGAATCTGTTATTGACCCAGAAAGATACAGTGGGAGTGTACAGAT	648	TCAGAGATGCTGGCTGACAAATCAATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	2444
T F L R R L V G K L F I D P E Q I O V G L V Q Y	187	S X D C G P O N E C V T D L V L O V H M D I R G	787
GGGAGAGCCCTGTACATGAGTGGTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	720	TCAGAGAGCCCTCATTTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	2520
G E S P V K E W S L G D F R T K E E V V R A A K	211	S R K A P F V V R G G R R K V L V S T T L E M R	811
AACCTCACTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	792	NAGCAAACTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	2592
N L S R R E G R E T X T A Q A I M V A C T G G F	235	K E N A Y N T S L S I I F S R H L P A V A H G N T F	835
AGTCACTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	864	CAGAGAGAGCCCTCAATAAGGTGGAATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	2664
S Q S H G C A P E A A A R L L V V V T D G E S H D	259	Q R E S P I K V E C A A P S A H A R L C S V G H	859
GGAGAGAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	936	CCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	2736
G E E L P A A L K A C E A G R V T R Y G I A V L	283	P V F Q T C A K V T F L L E Y E F S C S S L S	883
GGTCACTACCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	1008	CAGTCTTGGAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	2808
G H Y L R R R Q N D P S S F L R E I R T I A S D P	307	Q V F G K L T A S S D S L E R N G T L O E M T A	907
GATGAGGATTTCTTCAATGCTCAGAGATGAGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	1080	CAGCT	2880
D E R F F F H V T D E A A L T D I V D A L G D R	331	Q T S A Y I Q Y E P H L L F S S E S L S	931
ATTTTGGCTTGAAGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	1152	GTTCACTCATATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	2952
I F G L E C H A E N E S S Y G L E N S Q I G F	355	V H P Y G T L P V G P G P E F K T T L R V Q H L	955
TCCTCATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	1224	GGTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	3024
S T H R L R K D G I L F G M V G A Y D V G G S V L	379	G C Y V V S G L I I S A L L P A V A H G N T F	979
TGGCTTGAAGGAGCCACCCCTTTTCCCTGCAATGGCACTGGAGACGAGTTCCTGCTGCTGCTGCT	1296	CTATCACTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	3096
V L E C G H R L F P P R M A L E D E F P P A L Q	403	L S L S Q V I T M H A S C I V O H L T E P P G P	1003
AACATGAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	1368	CCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	3168
N H A A Y L G T S V S S M L L A G G R R L F L S	427	F V H P E E L Q H T M R L M G S N T Q C Q V V R	1027
GGGCT	1440	TGCT	3240
G A P R F R H R G K V I A T O L K K D G A V R V	451	C H L G Q L A K G T E V S V G L L R L V H N E F	1051
GCCAGAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	1512	TTCCAGAGCCCAAGTTCAAGTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	3312
A O S L O G C E O I G S Y F G S E L C P L D T D R	475	F R R A K P K S L T V V S T F A G C T G G A A C C A A C A G C A G T G C T	1075
GATGAGCACTGATGCTTACTTGTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	1584	CTACAGTCACTGAGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	3384
D E T T D V L L V A A P H F L G P Q N K E T G R	499	L O L T E A S R M S E S L L E V V O T R P I L I	1099
GTTATGCTATCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	1656	TCCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	3456
V Y V Y L V G O O S L L T L O G T L O P E P P Q	513	S L V I L I G S V L G G L L L L L L V F C L W	1123
GATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	1728	AAGCTTGGCTTCTTTCCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	3528
D A R F C F A H G A L P D L H O D G F A D V A V	547	K L C F P A H K K I F E E E K R E E K L E O	1145
GGGCT	1800	TACAAATAGGCTTACAAAGTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	3600
G A P L E D C H O G A C L Y L Y H C T O S G V R P	571	GCTGATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	3672
		AAGCATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	3744
		CAGCT	3816
		TATCCCAAGATCAATATTTTTCCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT	3888

FIGURE 6

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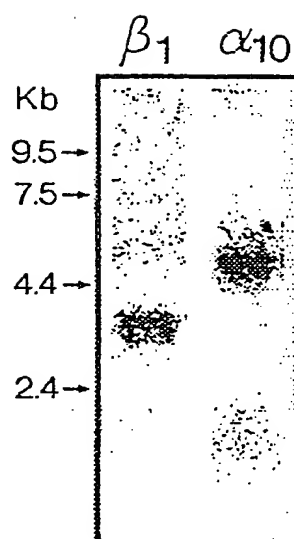
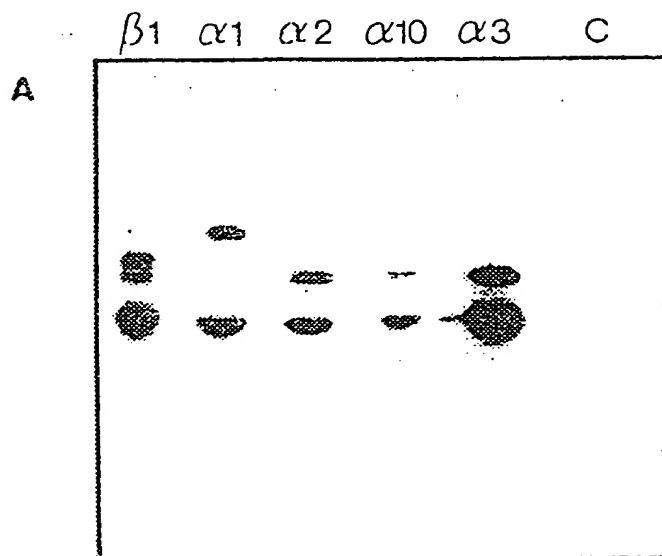


FIGURE 7

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B IP: $\alpha 10$ $\beta 1$
 Blot: $\beta 1$ $\beta 1$

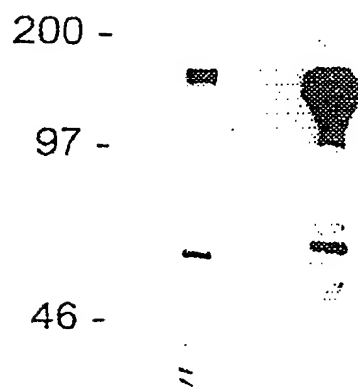


FIGURE 8

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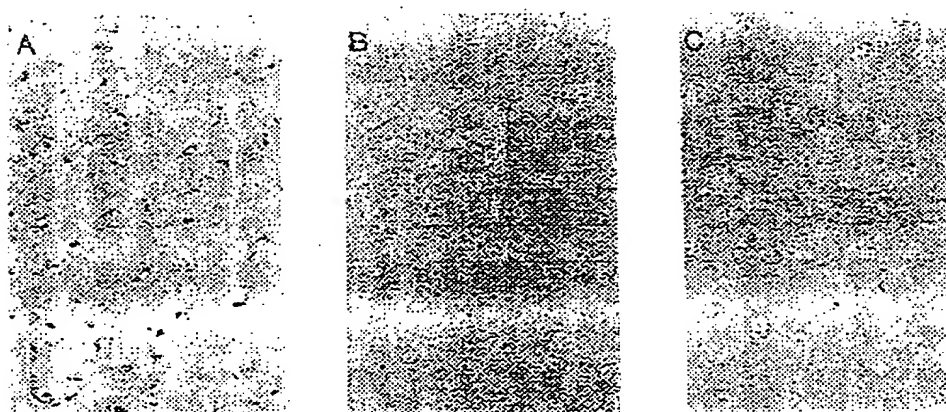


FIGURE 9

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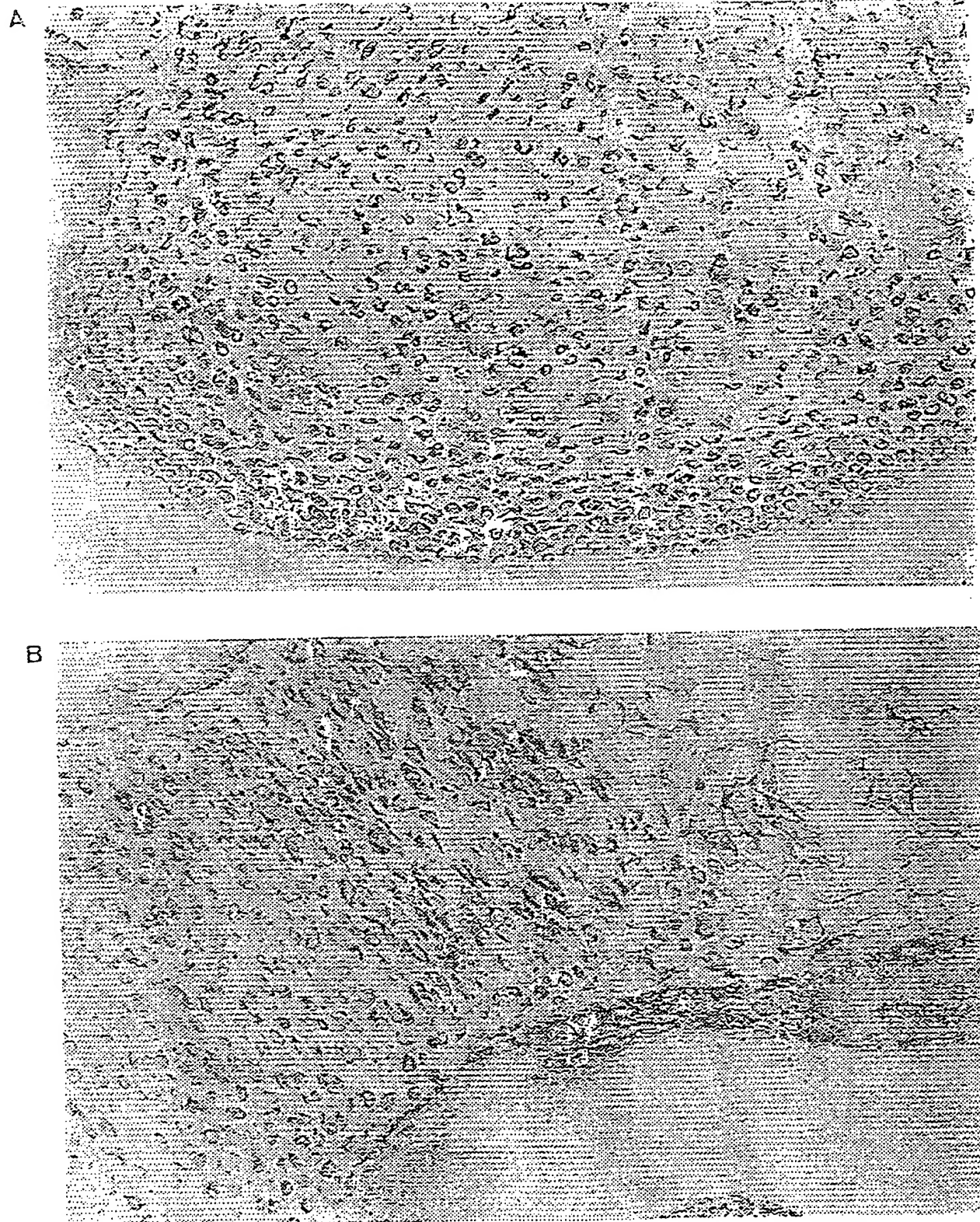


FIGURE 10

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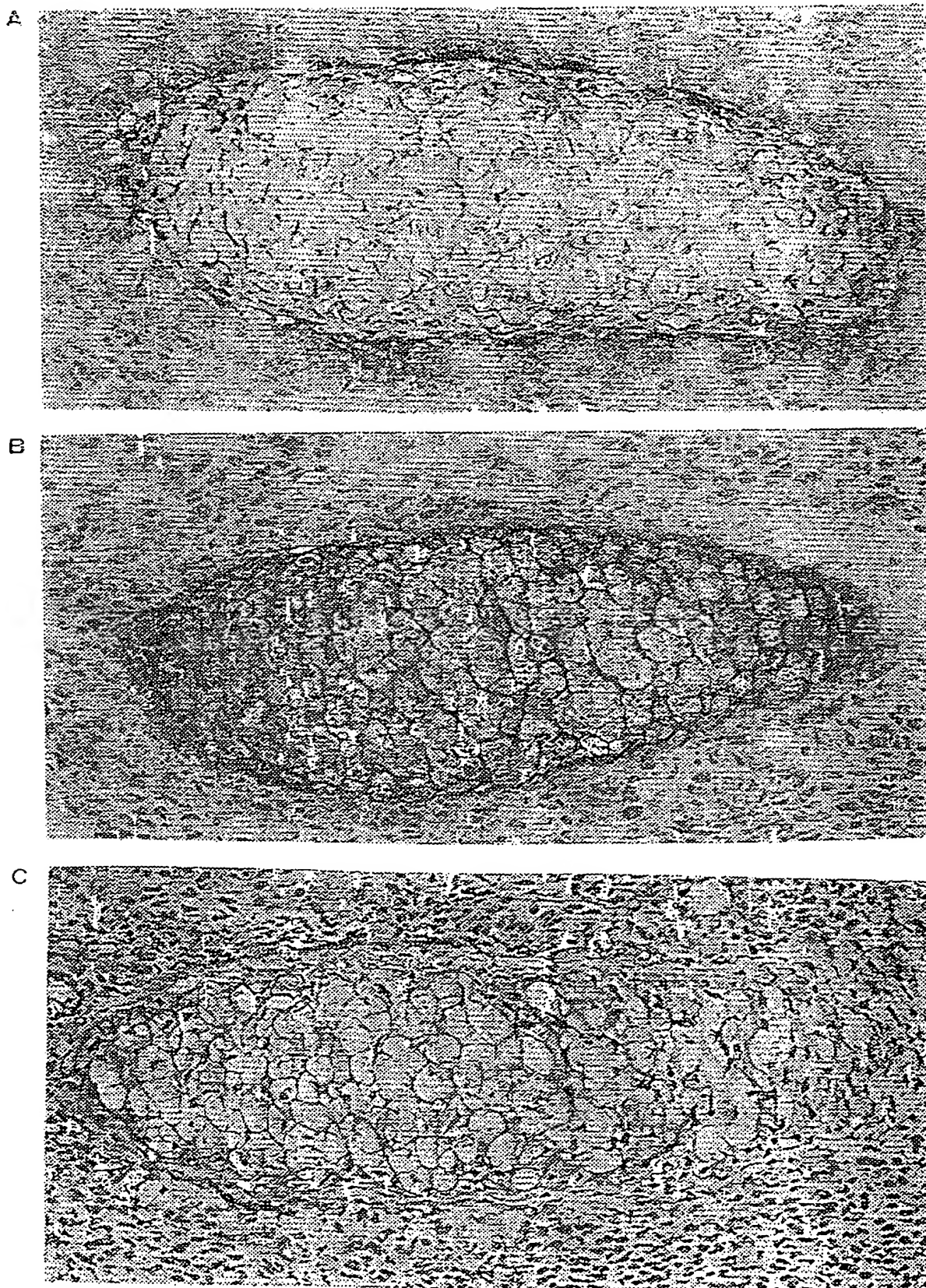


FIGURE 11

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Human RNA Master blot

Tissue	$\alpha 10$ expression	Tissue	$\alpha 10$ expression
Aorta	++++	Thyroid gland	-
Trachea	+	Salivary gland	-
Lung	++	Spleen	-
Fetal lung	++	Fetal spleen	-
Kidney	++	Thymus	-
Fetal kidney	(+)	Fetal thymus	-
Heart	(+)	Peripheral leucocyte	-
Fetal heart	++	Lymph node	-
Spinal cord	++	Appendix	-
Mammary gland	(+)	Placenta	-
Bone marrow	(+)	Whole brain	-
Small intestine	(+)	Fetal brain	-
Skeletal muscle	-	Amygdala	-
Liver	-	Caudate nucleus	-
Fetal liver	-	Cerebellum	-
Colon	-	Cerebral cortex	-
Bladder	-	Frontal lobe	-
Uterus	-	Hippocampus	-
Prostate	-	Medulla oblongata	-
Stomach	-	Occipital lobe	-
Testis	-	Putamen	-
Ovary	-	Substantia nigra	-
Pancreas	-	Temporal lobe	-
Pituitary gland	-	Thalamus	-
Adrenal gland	-	Subthalamic nucleus	-

FIGURE 12

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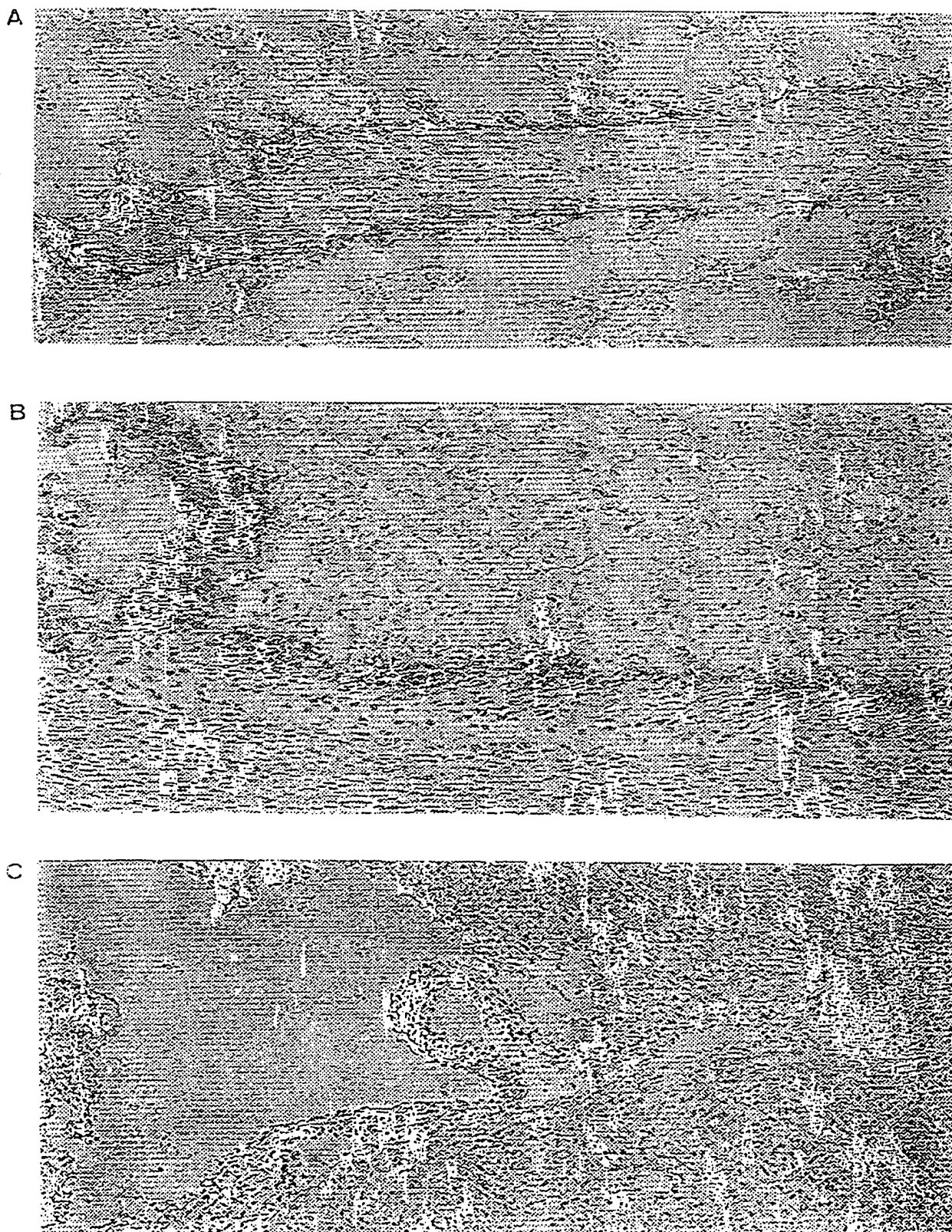


FIGURE 13

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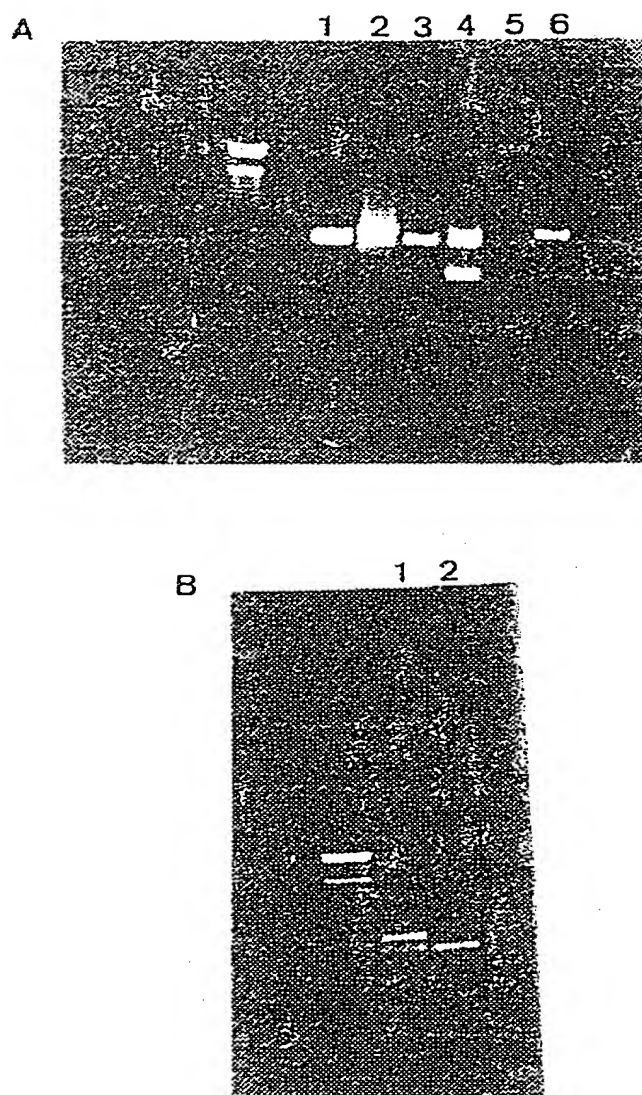


FIGURE 14

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TGNTHMFKCHCAGCAHQHWSAKXNCCGAKGGTKGKGVAAVGTGACARAGCTNGHNAARANGAAGTATGACCCWGTGGCC 80
 ? ? ? ? R ? ? ? P ? V ? ? ? D ? A ? ? K ? K Y D ? W A
 V ? ? ? H ? ? ? ? R ? ? ? ? V T ? L ? ? K ? ? S M T ? G
 ? ? ? ? T ? ? ? ? ? G ? ? ? . Q S ? ? K ? E V . P V G
 CRAGATAGMKAMDAAGCNGHSAGKTRAMGGACGATGGNCCHGCCAAVCGABWGGNAAHBTBGGCCHWDCAHNGTCCAAATK 160
 ? I ? ? K ? ? ? ? G R W ? ? Q ? ? G ? ? Q ? ? P N
 P R . ? ? ? S ? ? ? ? ? D D G P A ? R ? ? ? ? A ? ? V Q ?
 ? D ? ? ? A ? ? . R T H ? ? P ? ? ? ? ? ? ? S K ?
 SANKTCS CAGGAACCHACCGAHTGGCTCGCARCCCDTAGGGATCAGGKACGATGRCTCSCCGRNSKACTCSGNKTGATWA 240
 ? ? ? R N ? R ? O S ? Q P ? ? G I R ? D ? S P ? ? S ? . ?
 ? ? ? G T ? G ? A R ? P . G S G T M ? ? R ? ? T ? ? D ?
 ? ? S Q E P T ? W L A ? ? R D Q ? R ? L ? ? L ? ? I ?
 ATCGDNWGTGCGMAGGCGGCGGAATTWAAAAGTANTGGTHGAMAKATGCGVMGGAWATGATRRGTGACTVTHVMGGVAK 320
 I ? ? R ? A ? E L ? S ? G R ? H ? R ? . ? V D ? ? G ?
 S ? ? V G R R R A N ? K V ? V ? ? ? ? G ? D ? ? T ? ? R ?
 N R ? ? ? G G G ? ? K ? ? W ? ? ? ? ? ? ? M ? ? R L ? ? R ?
 VTAKSGGTACAGGCGAAKACARGRAKGTGTCTGAGGAADTCAGHAGGACAAHMTTGGCGAAGTCMGGACTTAOKATRGAT 400
 ? ? Y R R ? Q ? ? V . G ? Q ? D ? ? A E V R T . ? ?
 ? ? G T G E ? ? ? ? V S E E ? ? R T ? L P K S G L ? ? D
 ? ? V Q A ? T ? ? ? C L R ? S ? ? G Q ? C R S ? D L ? ? I
 ACCGAANCKTRGATCTTAMADGGGGGNKAGCGAGTGCSTAAACCVARATRGONSWGTCTACTTMAACNCCAAGNGDGGACA 480
 Y E ? ? I L ? G C ? R V ? K R ? ? ? ? L L ? ? Q ? ? T
 T ? ? ? S ? ? G ? S E C ? N ? ? ? ? ? V Y ? N ? K ? G H
 R ? ? D L ? ? G ? A S A . T ? ? G ? S T ? T P ? ? D
 TTTACTAGASGAGGAGAGTAGCCAGATCACDTGAGATGATCTAAKGTGGGGTCCCGTTGCCAGTATATGAGAGGAGTGGT 560
 F L R ? G E . P D H ? R . S ? V G S R C O Y M R G L V
 I Y . ? R R V A R S ? E M I . ? G V P L P V Y E R T G
 TCGGCAGACATWGATGCTCTTTGCTGACTCAGATATTGTTGCCVTGAGKATGATCAGATACGATCTGWTGTCCCTCATCA 640
 R Q T ? M L F A D S H I V A ? ? M I R Y D L ? S L I
 F G R H ? C S L L L T H I L L P . ? . S D T I ? C P S S
 S A D I D A L C . L T Y C C ? E ? D Q I R S ? V P H H
 TGAATSTGRGCCGTGATGCTAATGAGATTCCGCTATGATGGAACAAGAGACTTMTGCTACAGCAGGCGAATGAAGGTTTC 720
 M N ? ? R D A N E I R L . W N K R L ? L Q O A N E G F
 . ? ? A V M L M R F A Y D G T R D ? C Y S R R M K V S
 E ? ? P . C . . D S P M H E Q E T ? A T A G E . R F
 TAGAGTAGGAGTCTCAGGAGGAGAGAACTGTGGACCTGAGGACCAGGACTCCAGGAGGAAGTGWCCACAAGTGGCTT 800
 . S R S L R R R R E T V D L E D Q G L O E E V A T T G L
 L R V G V S G G E R N L W T P G R T R D S R R K ? P O L A
 L E . E S Q E E R N C W T P G G P G T P G G S ? H N , W L
 GHAGTTTTCGGCTCCGATCCTCATACWGGCTCGTCCTTVGAGTTATCCCCCTCTCTTGCTGGATGGCTCAGAAATGCCCTG 880
 ? F R L R S . Y ? L V L ? V I P L S C W M A Q K C L
 ? S F G S D P D T G S S ? E L S P S L A G W L R N A W
 ? V S A P I L I ? A R P ? S Y P P L L L D G S E M P G
 ACCTTTTCATCCCCACTGGACAACTAGGCGTCTGGCGTTGTGGCCCTGGGATTGTGGGGCTGTGTGGCCTCATATCCTC 960
 D L F S I P T G Q T R R L A L W P W D C G A V W P H I L
 T F S S P L D K L G V W R C G P G I V G L C G L C V S Y P
 P F H P H W T N . A S G V V A L G L W G C V A S Y P
 CATTCTGTCTATTCTCACCTAATCTGTCCCTGGNTACGACTCAAGCCCYGACTGACAMTGTGGTACAAGATAAGGAGCG 1040
 H S V Y S H P N L S L ? T T Q A ? T D ? V V O D K E G
 I L S I L T L I C P W ? R L K P ? L T ? W Y K I R R
 P F C L F S P . S V P G Y D S S P D . ? C G T R . G G
 AGCCCCAGGTGGGTGAGATGGAAGCTGAGATGGTNCACCTGTGTGCCACCTCATTGTAAATCAACTNCCTTGACTGAAGTT 1120
 A O V G E M E A E M V H C V P T S L . F N ? L D . S
 E P R W V R W K L R W ? T V C ? P H C N S T ? L T E V
 S P G G . D G S . D G ? L C A ? L I V I Q L P . L K L
 AAAATCCAGATCCYTACGGATGAGGGGAAGAACTGCCAAAGACGGGTACAGGAAGGCAGTGCTAAGGGAAGGCTCCTGCA 1200
 . N P D P . G . G E E P A K D G S G R O C . G K A P A
 K I O I ? R D E G K N L P K T R V H K A V L R E G S C
 GGCCTCTGCAGTTGGACTTCATTGAGTCCCATTTGTCAGAATCTCATAGCTCTTCCCYTATCTCTCTGCTCTGAGTCTAG 1280
 G L C S W T S F S P I A R I S . L F P L S L C L E S S
 A S A V G L H S V P L P E S H S S S ? Y L S L S L
 R P L Q L D F I Q S H C Q N L I A L P ? I S L S . V .
 TTAAGAATTTGTTACCGGAGACAGAATTCTCTTTCTTAGCCCTCCTGGCCAGATATTTAAAGGACGGGGCTGGTTACTT 1360
 . K F V T C D R I L F L S L A R Y L K G G W V T
 V K H L L P E T E F S L S W P D I . K E G G G L L
 L R I C Y R R Q N S L S . P P G Q I F K R R G V G Y F

FIGURE 15a

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TTTGGTAGCGGAAGCTTAAGTTATGGATAGCAAAGTGCTAATTGTATCTTTTTTTCTGAAAGCTCATGTAGCATTTTTC 1440
F W . G K L K L W I A K C . L Y S F F L K P H V A F F
F G K G S L S Y G . U S A N C I L F F . H L H . H F S
L V G E A . V H D S K V L I V F F F S E T S C S I F
TTCCCTTCCACCTCCATACTTTCCAGGCTTCATTTCATGCCCGGCTCTCTTCGCTCAGACCGGTGAGGCTGTTGA 1520
F P S T L H T P P G F I S C P A S L R S H R C R L F E
S L P P S I L S O A S F H A R R L F A H T A A G C L
L P F H P P Y F P R L H F M P G V S S L T P L O A V .
GGCTTCTCCCTCGGCTCTGCTCAGCAGACTGCCTCCACACTTTCCAGTTTCTCGCTACACCTTGATATTAGAGTTTCT 1600
A S P L G L P O O T A S T L S S F C V H V D I R V S
R L L P W V C L S R L P P H F P V S A Y T L I L E F P
G F S P G S A S A D C L H T F O F L R T R . Y . S F L
TCCCCACTTGGCTCTTGTCTTTCTGACTAGCCAGGCTGATGCCATGTCTGGCTCTTCTCTGTAATACTGTACAATG 1680
F P T W L L L F L . L P R L H F C L A S S C K Y C T H
S P L G S C S F S D Y P G . C H V W P L P V N T V Q .
P H L A L A L S L T T Q A D A M S G L F L . I L Y N .
ATTCTATGTAATAACTGGTCTTGGCCACAGAGCAAGCCCTTCTAGCTAACAATAAAGATCAAGTTTGTCTCAC 1760
I L C K . L V L A H R A S K P S R L T N . R S S L L T
F Y V N N W S L P T E O A S L L G . Q I K D Q V C S
D S H . I T G F C P O S K Q A F . A N K L K I K F A H
TGACTTTTTATTCAATTCAAGATGGCGGGGGTGGGTGGGGGGGCGATTGCTCTTTCTACTGTGGTACCTAGGCAG 1840
D F F I O F K H A G G G V G G R I A C F H C G T . A
L T F L F N S R W R G V G W G G G L P V F T V V P R O
L F Y S I Q D G G G W G G G A D C L F S L W Y L G R
CGCTGAAGCTCTGAGCTCCCTGCTTTAGGCTTCTGAGTAGCCTACAGTGAGTGTACTGTGTCCAGCTGCTCGTTGACA 1920
G L K L . A P L L . A S E . P T V S V T V S S C S L T
O . S S E L P C F R L L S S L O . V L L C P A A R . H
A E A I S S P A L G F . V A Y S E C Y C V Q L L V D
TCTGGTCTCTCATGCTGCTGCTATGTAAGCCITAGCTCTCTGACTGTGGATGGCTTTCTTGGCGTTAGCAGCTAACAT 2000
S G L S W S G H C K P . L S D C G W L S L A L A A N H
L V S H G L V I V S L S S L T V D G F P W R . O L T
I W S L M V W S L . A L A L . L W M A F L G V S S . H
GGTTACAGGATTTCACTGAAAAATTTAAATGTTGGGGGAAAGGTGGGGACACACCATAATGGTCCCAATTCAAAACAATCC 2080
V T O F H . N F K C W G K G A D T P . W S O F K T I
W L O D F T E N H V G G K G V R T H H N G P N S K Q S
G Y R I S L K I . H L G E R C G H T I H V P I O N P
GTCAACAGCTCAAGTTACGGGTGAGATGTTTCAACCAAGTAATTATCTTGACACCACAAAGCACACCTGTCTACAG 2160
R E T A S S . G . D V F N Q S N Y L D T T K H T C L O
V K O P Q V R G E H F S T K V I I L T P O S T P V Y R
N S L K L G V R C F O P K . L S . H N K A H L S T
GCAGTGACTCCCCAAAGCTATTAGACACACAACAAGCATGACCATAACTCAGTGGATTGGCAAGGTACACAGTAGGAC 2240
A V T P Q K L L D T Q Q A . P . L S G L A R S H S R T
Q . L P K S Y . T H N K K H D H N S V D W Q G H T V G
G S D S P K A I R H T T S M T I T Q W I C K V T Q . D
TGCCCTTACACAGTAGGTAGGAAAATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2320
A L H T V G R K H L L S L L S A V I L H I P C . D .
L F F T Q . V G K C C H C C Q L L F C I S H V K I N
C P S H S R . E N A C A V T A V S C Y F A Y P M L R L I
AAGGCAAAAAATATTGCTCTTAAGTCTTCTGTTTCTGTTTCTGTTTCTGTTTCTGTTTCTGTTTCTGTTTCTGTTTCT 2400
G K K Y C L . V L L S V P N W R K L L N K . T V H K
K A K N I V S K S Y F L F O T G G N Y . I N K P C I K
R Q K I L S L S P T F C S K L E E I I E . I N R A .
AGTAGCCTCAGAAAGGCTCAAAATTTGTGTTTTCTTTGAATATTAGCTGAGCGCTCCAGGGGGCAGCACCAAGGTAGAGA 2480
S S L R K V Q N L C F L . I L A E A S R G O H Q G R E
V A S E R V K I C V F F E Y . L R P P G G S T K V E
K . P O K C S X F V F S L N I S . G L O C A A P R . R
CCTGGACTAAGGCT 2560
L D . G C S V F L S W A P H S S L P P P L F F H P T
S W T K A A L C S C P G L F T A P F H H H S H S I O L
A G L R L L C V F V L G S P Q L F S T T T P I P S N F
TATTTTTAGCTGCCAGTGGAGCGGGGAGGATAGGAGGAAAGTAACGAAAAAGGCAAGGAGAGGGAGAGGCAACTCA 2640
L F L A A S G R C O D R R E S N E N S Q C E G Q S N S
Y F . L P V C G G R I G G K V T K T A K E R D R A T Q
I F S C O W E G A G . E G K . R K Q P R R G T E Q L
GAGCCTCTCGGACTGGAGCGGACAAGCGCCCTGAGTCTCTCTCATCCCTCAGCTGCTGCTGCTGCTGCTGCTGCTGCT 2720
E P L G L D R T S A H G V S L H F S P A P A P G V A D
S L S D W T G Q A P H E S L S I P H L L L L L A T L
R A S R T G P D K R P W S L S F S L T C S C P W R ? .

FIGURE 15b

CAGGTGAGGAGGAGCAAACCTTGGTTTCTCTCGGAATGGGAAGTTAGTGTGGATTGTTTATAATTTGGGACCAATTATGCTATAA 2800

R . G K Q T W F L L G H E V M W I V Y N W D H Y G .
U G V E R E A N L V S A G N G S Y V D C L . L G P L W L K
ATCTYGGCGGCGCTCAGGTGGAGGTTAATACCGATGCTATATTTCTGTGTGCACATCATGTTCTTAGACACCCAAATGG 2880

N L A G A Q V G G . Y R C Y I S C V H S C S . T P K W
I ? ? R A L R S E V N T D A I F P V C T H V L R H P N G
I ? ? G R S G R R L I P H L Y F L C A L M F L D T Q H

CAGTGGGCAAAACTTCTCTGGCTTGTACCTCATTATCTAAACCTTTGTACCTAATTATCTAAACCTTGGTCTCTAACT 2960

Q W P K L P L A C T P H Y L S K P L Y L I . N L G P F K L
S G Q N F L W L V P S H Y L N L C T . L I S K T L V L N
A V A K T S S G L Y L I I . T F V P N Y L K P W S .

CCACAGACATGAGGGCAGAGAAAAGAGACGTGTCTCTCATCTCCATTCCGTTACACTGATTCCCTACTTCCCTGCTTCT 3040

H R H E G T E K R R V S H L P F G Y T D S Y L P C F
S T D M R A Q K R D V S L I F H S V T L I P T F P A S
F P Q T . G H R K E T C L S S S I R L H . F L P S L L L

CCCTGCTATTGGTCTCTTGGTGGCTGAGGCATAATGCGCTTACTATGTGGTCAGAACTCTGGGTTCCGCTAACGACCG 3120

S L P L V L L G A . G T I A L L C G G O N S G F A . R P
P C H W C S L V P S A . L P Y Y V V R T L G S P N D R
P A I G A P W C L R H N C L T H W S E L W V R L T T

AGCTACAGTTTCTGGTCTCATAGCCCTGCCAAATTTCTGGATTAAAAAAGGCTCACATATAAAATACCTTTTCTGA 3200

S Y S F W S H S P A N F L D . K K K A H I . N T F S E
E L T V S G L I A L P I S W I K K R L T Y K I P F L
E L Q F L S . P C O F P P L K K K G S H I K Y L F .

AAATGAGCACAGTGTGAGTTGAAGTTAGATTTTGGCGGATGGAGGGTTGCTTGGATGCAAAGAGCAAGACAGTAGAGAAG 3280

N E H S V S . S . I L G D G G L L G C K E Q D S R E
K M S T V . V E V R F W G H E G C L L D A K S Q T V R E
K . A Q C E L K L D F G G W R V A W M Q R A R Q . R R

AGAATCATGGGAGGGATAAGAGGCTGGAATTTTCTCTGCTAGTCCCTATAATCTTTCTTCTTAAATACAGCTCTG 3360

E N H G R D K R L E F F P A S A L . S L F P K I T A L
R I H G G I K R L E F F P A S A L . S L F P K I T A L
E S W E G . E A G I F F P C . C P I I F V S . N N S S

ATTTTATGGGAATTGGGGTCAGGAGAAAAGGAATCAGTAGGCACAGATGGGACCCCAAGCGTGGACTAAAGTTTGAGGAAA 3440

I L W E L G S G E R N O . A O M G P O A W T K V . G N
D F Y G N W G Q E K G I S R H R W D P K R G L K F E E
D F H G I G V R K E S V G T R D G T P S V D . S L R K

CTATGGGAGTAGGCAAGGGGTTTGTAAAGTGGATGAGATGACGAGATTGTGCTTGGGGGGGATCTTGGGGGTCATAGG 3520

Y G S R O G V F V R W M R . G D C G G G E S W G .
T H G V G K G C L . G G . D E E I V V G G S L G G D R
L W E . A R G V C K V D E H R R L W W G G V L G V I G

ACCCTTAACAGGGATAGATGGCAAACCTGTGTGTGGGCAGGCCGTTGCTTCCACCCACTTAATTAGCCTTGAGGTTGGCAG 3600

D P . Q G . M A N C V W A G R W F H P L H . R . G W Q
T L N R D R W Q T V C G G A G O S T H L I S V E V G R
P L T G I D G K L C V G A F V V P P T . L A L H L A

GGCTGGAAGGAGCCAGCACTCTCAACCTTGGAGAAAGTGCAAGTGTGACAAAGAAACAGAAAGAGGAGACACCCGGCC 3680

G W K R E P A L S T L E K V Q V . Q E E T E R G O D T R A
A C K S Q H L S O P W R K C K C Q D K K K O K E E T P G
C L E G A S T L N L G E S A S V T R R R N R R H P G

AGGGAGCTCTTGCATCGTTTCTTCCCATGGCCCTGGGTTTGGGAAGAATTAGGAAAGGGTGGTGACTCTGCATCCTCA 3760

G S S L P S F L P H A L A L G R I R K K G W . L C I L
Q G A P C H R F F P P W L W E E L G K G G D S A S
R E L L A I V S S H G P G F G X N . E R V V T L H P O

GAAAAGCCCTCTCTCCCTCTTTGGACTCTCGAGGCTTAGAGAGGAGAAATGTGTAGGAGGAATGATGTGGAAGAGTAAGT 3840

R K A L S P S L D S R G L E R R M C R R N D V E R V T
E K P S L P L W T L E A . R G E C V G G M H W K E . L
K S P L S L F L G L S R L R E N V . E E . C G K S N

TGACCTATCCAGATGTCTGTGAATGACATTTCAGGAATGAGAATGGAAATACAGCTGTGCTTCAGCATGCCCGAGGGC 3920

P I O M C L . H R F Q E . E W K Y S C A S A W P R A
D L S R M C V C E . D F R H E N G N T A V L O H G P R A
L T Y P D V S V N E I S G H R M S E I Q L C F S M A E C

CTTAGGATCCCTCACCCTCACCCTCAGGAAAGAGAAATCATCCAATCATCCCACTGGGGTTCTGAGGACATGACATTGAC 4000

L G S L T P P T P H C E N R H I P F G V L R T . H .
P . D P S P P P H R K N R I Q S S H L G F . C H D I D
L R I P H P H P T G R E S S N H P T W G S E D M T L T

ACAGAGCCAGGAGCTGAGATAGAAACACTCCCTGCTGTGTGTCTCCCACTAAGCCTCAACGACTCTTCATTAAGTGAT 4080

H R A G E L R . K H S L L S C L P L S L T S P S L T D
T E O R E S . D R N T P S C L V S P H . A S P V L H . L I
Q S P R A E I E T L P P V L S P T K P H Q S F I N .

SUBSTITUTE SHEET (RULE 26)

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TGGTGGATGCTAATTATGATCCTCACCCCTCAGGTCTCTGCTCCCTTTAATCTGGATGAACACCACCCACGACTCTTC 4160
 W W M L I M I L T P Q V S A F P L I W M N T T H D S S
 L G G C . L . S S P L R S L L P L . S G . T P P T T L
 L V D A N Y D P H P S G L C S P F N L D E H H P R L F
 ACAGGGCCACAGAGGCCGAATTTGGATACAGTGTCTTACAGCATGTTGGGGGTGGACAGCGATGGTGAGAGGGAAAAACA 4240
 Q G H Q R P N L D T V S Y S M L G V D S D G E R E N
 H R A T R G R I W I O C L T A C W G W T A H V R G K T
 T G P P E A E F G Y S V L Q H V G C G Q R W . S G K Q
 GAGGACCGTGGGATCGGGACTATGCACTCACTGATAAAGGGGAGGACCGTCCAAGCTGGCCTTTGAAAGTGCCTGGGGC 4320
 R G P W D R D Y A L T D K G E D R S K L A F E S A W G
 E D R G I G T M H S L I K G R T G P S W P L K V P G A
 R T V G S G L C T H . R G G F V Q A G L . K C L G
 TCCATGACGTCTCATGCACTCTCCCTCTCACTATACTAAGGACCATGCTCACCGGATCTTTATATCCATATTCTCCTTCC 4400
 S M T S H A L S L S L Y . G P C S P D L Y I H I L L P
 L H D V S C T L P L T I L R T M L T G S L Y P Y S P S
 AGGATGCTGGTGGGTGCCCCCTGGGATGGGCCATCAGGTGACCGGAGAGGGGATGTTTATCGTTCCTCTATAGGGGATT 4480
 G C W W V P P G M H Q V T P E R G M F I V A L . G D
 Q D A G C P L G W A I R . P E R G C L S L L Y R G I
 R M L V G A P W D G P S G D R R G D V Y R C S I G G F
 CCACAGTCTCATGTACCAAGGCCACCTGGGTAAAGAAAGCGTCACTTTCCCTGCTAATTCGTATGTTGACATC 4560
 S T V L H V P K A T W V R R S I T F P L L I P D V D I
 P O C S M Y Q R P P G . E E A . P F P C . F L M L T S
 H S A P C T K G H L G K K P D L S P A N S . C . H
 TAGTAACCTCTGACCCCTTGACCTTGTCTCAATGACCCGAACTAAAGAAAGCGAACTATGACCCCATGACTTCATTCT 4640
 . . L . P L G P C L Q . P . T K E A E L . P H D F I L
 L V N S D P L D L V F N D P E L K K P N Y D F H T S F
 L V T L T F W T L S M T L N . R S R T H T P . L H S
 CTTCTACCCCTTCTCCCAACGAGGTGACTATCAACTTGGAAATTCCTCTCAGCCTGCTGTGAATATGCACCTAGGGATGTC 4720
 F Y P S S H Q V T I N L E I P L S L L . I C T . G C
 S S T P L P P T R . L S T W K F L S A C C E Y A P R D V
 L L P F L Q T F G D Y Q L G N S S Q P A V N M H L G M S
 TCTACTAGAGACAGATGCTGATGAGGCAATTGATGCTGAGCTGAAAGAAAGGGCCTCAGAGGTTTACAGCAGGGAAGAGAG 4800
 L Y . R Q M L M G D S W . A E R R A S E G S Q Q G R E
 S T R D R C . W G I H G E L K E G P Q K V H S R E S
 L L E T D A D G G F M V S . K K G L R R F T A G K R
 CATTATGGTATCTGGGCAGTGGTGGCTTGGGCCCTTTCATCCAGTGTCTGAGGAGCAGAGTCAGGCCCTGATCTACAGAGT 4880
 H Y G I W A V V A W A F H P S V F L E A E S G L I Y R V
 I M V S G O W W L G P I P V F W R O S Q A . S T E
 A L W Y L G S G G L G L S S Q C S G G R V R P D L Q S
 GAGCTCCAGGACAGCCAAAGGCTATGAGAGAAACCCCTGTTTGA AAAACCCAAACCCAAACCTAACCAACAACAAC 4960
 S S R T A K A H R N P V L K N P K P K L T K Q Q O
 . A P G Q P R L C R E T P F . K T O N Q N . P N N N N
 E L Q D S Q G Y A E K P C F E K P K T K T N Q T T T
 AGAAAAGCACCGTGGTAAGGAAATTAGTCTGTATAGAAGAGATCAAGGAATTCAAAACCTAGAGAGCAAGGCAGGGT 5040
 Q K K H R G K G N . S V . K R O G I O N P R E O G R V
 R K S T V V R E I S L Y R R D K E F K T L E S K A G F
 E K A P W . G K L V C I E E T R N S K F . R A R Q G
 CCCCATGGAGTGGTCTCCATCTCTCTTTAACTAGGTGTGTGTTCCGAGAGGCCCTCTCAAGCCTGGGGATAACTATTTC 5120
 P H G V V S I S L L T R C V F R E A L S S L G I T I S
 S P M E W S F S L F . L G V C S E R P S O A W G . L F
 S P W S G L H L S F N . V C V P R G P L K P G D N Y F
 TCCTATCCACCCAGGCGTGTGCCCTCTTTGGTCTCGTGCCTGGCGGAGCTCTGTCTCAOTTCTGGAATATGTGCCGT 5200
 P I H P G L C P S L V S C L R Q L C L Q F W N H C P
 L L S T Q A C A P L W S R A C G S S V F S S G I C A R
 S Y P P R P V P L F G L V P A A A L S S V L E Y V P V
 GTGGATGCTTCATTCCGCCCCCAGGGAAGCCTGGCACCCACCCGCAACCTGAGCCAGTGGAAAGGCCCTGGAAAGCTCAG 5280
 C G C F I P A P G K P C T H R P T . A S G R A L E A O
 V D A F R P O G S L A P T A Q R E P V E G F P G S S
 W M L H S G P R E A W H P P P N V S Q W K G P G S S
 TTCCAGATAGGGATGCTGGGTGGGAAAACTAGGACAAAGACTTCGTGGAGCGCTGTCATGGCTATCCTCATCTCCC 5360
 F P D R A G W E K L G Q R L G G G S A W L S S S F P
 V P R . G C W V C K T R T K D L W E G L H G Y P H H S
 V P R . G C W V C K T R T K D L W E G L H G Y P H H S
 AAGTGTGCTTGCAGAAGAGGCTCTGTTTGCTAACTGATTAGAATTAGAGTCTTACGAGAGCCCTCAAGACACCCAGGAT 5440
 S V L A E E A P V C . L I R I Q T P . E S L K T P G
 Q V C L O K R L L F A N . L S F R L L R R A S R H O D
 K C A C R R G S C L L T D . N S D S L G E P Q D T R I

FIGURE 15d

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CTGGTTTTACCAACTTAAAAACAAAACAAAACAGCATATCCTGTGCACAGCCTATCCCTCATCCATCACGTCCTCCAT 5520
S G F T N L K T K Q N S I S C A Q P I F H P S R V L H
L V L P T . K Q N K T A Y P V H S L S L I H H V S S I
W F Y O L K N K T K Q H I L C T A Y P S S I T C P P
ATCTTATTTTGTGGGTCTTAGATGCCAAGTCAGCACTCAGTTATTGGCTTCTCCCTCATGCCCTTTCATATACITTC 5500
I L F L W V L . M P S Q H S V I G F S P H A F H I L S
S Y F C G S Y R C Q V S T Q L L G S P L H P F I L S
Y L I F V G L I D A K S A L S Y W V L F S C L S Y T F
TTATCTACTGCCCTTTGGGAGATAGTCTTATGTAGCCAGCCTGTCTTGATCTTGGAAITTCCTTGCCTCAGCTTCTCA 5680
Y L L P F G R . S Y V A Q A V L D L G I C L P Q L L
L I Y C L L G D S L H . P R L S L I L E F A C L S F S
L S T A F W E I V L C S F G C P . S W N L L A S A S Q
GTCTCAAGTACTGGGATAATAGGCATGCATTGTCTGCCCTTTCCTGGAACATGCCCTCTGTGCCATTGTAGGCCA 5760
S L K Y W D N R H A L S A W P L L N M P S V A I G R A
V S S T G I I G M H C L P G L C . T C P L W P L V G H
S Q V L G . . A C I V C L A F A E H A L C G H W . G
TGAGTCAAACTACTGCCCTCCCCACACACACACAAAGGAGTGGCTCTCTAAGTGTTCATAGCACAGGGTACT 5840
V K Y C P P P P O H T H K R K . G S L S V P . H R V V
E S N T A L P H N T H T N E S E A L . V F H S T G .
M S Q I L P S P T T H T Q T K V R L S K C S I A Q G S
GGTAGGCCCTCTCGCTAGTCATATTTCTTTTACTCTGCCCATCTCTTCTTTCTTTGATTTCCACACTGGGGACCTG 5920
V G L S L V H I S F F Y S A H L F F L . F P H W G P
W . A S R . C I F H S F T L F I S S S F F D F H T G D L
G R P L A S A Y F I L L L C P S L L S L I S T L G T W
CGATAGTACTTTCTCGTAATTAAGAGAGAATTCCTTTTAAAGTGCCTGCATTGCAGCGTCTCTCGGACATTCTCCCT 6000
G I V L S W . L R E N S L L S A C I A A S S W D I L P
A . Y F P G N . E R I P F . V P A L Q R P P F G S L
H S T F L V I K R E F P F K C L H C S V L L G H S P
TGCTGACTACACCCACATCCTTCCATGTTTTTGTTCCTCACTATGCCCTTCTAGGCTGTCCACATACATGG 6080
G . L H F T S P H V F C F P S L C P P S R L S H I H G
A D Y T T P H I L P C F L F P I T H M P P F . A V F H T W
LTGTGCTCATTTGTTGGATGGCTCCAACAGTATCTATCCTGGTCAGAAGTTCAGACTTTTCCTTGGAGCGCTGGTAGGA 6160
C A H C F G W L O U Y L S L V R S S D F P S E A G R
D V V . V L D G E N S I Y F W S E V O T F L R R L V G
M S S L F W M A P T V S I P G Q K F R L S F R G W . E
AGACTGTTTCATCGATCCGGAGCAGATACAGGTAAGAGAAAGATATGTGGATAGGATTGGAGGAAAGAACTAAACACTCC 6240
K T V H R S G A D T G K R K I C G . D W R E R S K H S
H L F I D P E O I O V R E R Y V D R I G G K E V N T P
D C S S I R S R Y R . E K D M W I G L E G K K . T L
TGGACCTTGGATGTAAGCAGCCATGTCCAGCCTCTTGATGACACCCCTGGGACATTGTCTTCTACAGAAGTCTGCTCAA 6320
W T L G C K O P C P A S . . H P G T L S S T E L M L K
G P L D V S S H V C P L D D T L G H C L L L H S C S
L D P W M . A A M S S L L M T P W D I V F Y R T H A O
GAAGTGTGCAATTAACCTTACCAAAAAGTCACAAAATTTTCATAATGTTTGAAGTAAGTTTATGATTGTGTGGGGGGCCAC 6400
N C A I N L P K S H K N F I M F E V S L . L C G G G F
R T V Q L T Y O K V T K I S . C L K . V Y D C V G G F
E L C N . L T K K S O K F H N V . S K F M I V W G A T
ACTCAGAGCTTCCCTTTGCTGCTTGTATTTGCTTGGGCAATGCATGCCATGAGCTCCAAGTTAGACACACCTGTTCACTT 6480
H S E L P F A A C S C L G N A C H E L Q V R H T C S L
T O S F P L L L V V A W A M H A M S C K L D T P V H F
L R A S L C C L . L L G Q C M P . A A S . T H L F T
CCCCTTCATCGTGTGCGAGGTGGACACACCTGTTAGGGGTTCACTTCCCTTCATCCTTTGTGCTCCATCTTCTCTACG 6560
P L H R A A G W T H L L G V H F P F I L C A P S S L R
P F I V L Q V G H T C . G F T S P S S F V L H L L Y
S S S C C R L D T P V R G S L P L H F L C S I F S T
CTCTTCATACATCCCATGTGGGCACATGGTCTATTGTTCTCAGGTAGGACTCGTACAGTACGGGAGAAACCTGTGCGATG 6640
S S Y I P C G H M V Y C S O V G L V Q Y G E N P V H
A L H T S H V G T W S I V L R . D W Y S T G R T L C M
L F I H F H W A H G S L L F S G R T G T V R G E P C A .
AGTGGTCCCTGGAGACTTCCGAACAAAGGAAGATGTGTGAGCAGCAGGAACCTAAGTCCGAGCGGAGGGCGAGAA 6720
E W S L G D F R T K F E V V R A A R N L S R R E G R E
S G P H E T S E Q R K K L . E Q Q G T . V G G K G R E K
V V P G R L P N K G R S C E S S K E P K S E G G R A R
ACGAGAACCGCCCAAGCGATCATGGTGGCATCGTGAGACATTGTAAAGGGGTCTGTGTAGGGAGGAGGAAGGATCAGCAG 6800
T P T A Q A I M V A W . D I V K G S C E G G G R I S A
R E P P K R S W W H G E T L . R G R V R E E E G S A
N E N R P S D H G C H V R H C K G V V . G R R K D O O

FIGURE 15e

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GGAGAGGGAGAGGGTCTGGAGTGTAGTGTATACATCACAAGATGCTCTGGGCGCTTATCTTTATCTGCATGCCAGAAGTT 6880
E R E R V W S V V Y T S Q D A L G A Y L Y L H A R S
G R G R G S G V . C I H H K M L W A L I F I C H P E V
G E G E G L E C S V Y I T R C S G R L S L S A C Q K F

CGTGGAGGAAGGCTAGGTTGCTGTACCATACTCTCTTACTGTATTIGCATTTTATGGTGTCTGTGGGTGTATCTCTC 6960
S W R K A R L L S P Y S L L L Y L H F M V S V G V S L
R G G R L G C C H H T L S Y C I C I L W C L W V Y L S
V E E G . V A V T I L S L T V F A F Y G V C G C I S

CTTGTCTGTTCTGTTTCTGCACACAGAAGTCCATCTTTCTCTTCTACTCTGCGTCAATTCTGATACCTAGCTTCTCAA 7040
L V C S V S A H R T P S F L F Y S C V N S D T . L L N
L S V L F L H T E L H L S S T P A S I L I P S F S
P C L F C F C T Q N S I F P L L L L R Q F . Y L A S Q

CCACTCAGCCCTAGTATTCTTTCAAACATGACTCTAAACCTCTGGGAGGCTACATGACCTGACTGTCTTTATTCTCC 7120
H S R P S I L F K H D S K P L G R L H D L T V F I L
T T H A L V F F S N M T L N L W G G Y M T . L S L F S
P L T P . Y S F Q T . L . T S G E A T . P D C L Y S P

AGTTCCTTGATCTTGTCACCCCAAGTGTGCTGAATGAATCTATAAATAAATAGCTTGATACATTTTACACTGATGA 7200
Q F L D L V N P S V C . H N L . I N N A C T Y L H .
S S L L S T Q V F A E . I Y K . I M L V H Y T . D D
V P . S C Q P K C L L H E S I N K . C L Y I F T L M

CAGATTATTTATATGTTCCGTGCCATCTAAACAGTCAAGTTGTGACTCTGTGCCAGTTTGCATGCTAGATACTGTGGG 7280
Q I I L Y V P C H L N S Q V V T L C Q F A C . I L L G
R L F Y M F A I . T V K L . L C A S L H A R Y C W
T D Y F I C S V P S K Q S S C D S V P V C M L D T V G

GAATGGTGTAGAAGACATCTGACCTCAGTGAAGTGTGACAGTGTAAATACACTATACGGGCATGCGTGCATGCAAGCCT 7360
N G V E D I . P Q . T A D S V N T L Y C H A C H O A
G M V . K T S D L S E L L T V L I H Y T G M P A C K P
E W C R R H L T S V N C . Q C . Y T I R A C L H A S L

GTGTGTATGTGCATATGCACACACATACATATGACCATATAGCATTTCTTTATCTCTTCTTAGCACAGAAGGT 7440
C V Y V H A Y A H T Y I . P Y S I L L S L F L A Q K G
V C M C M H M H T H I Y D H I A F F Y L S S . H R R V
C V C A C I C T H I H M T I . H S F I S L S L S T E G

TCAGTCAGTCCCCGGGGGACGACCAGAGGCGCTAGGCTGCTGGTAGTTGTCACTGATGGAGAGTCCCATGATGGAGAG 7520
S V S P G G D D T Q R P L G C W . L S L M E S P M H E R
O S V P G G T T R G R . A A G S C H . W R V P . W R
F S Q S R G G R P E A A R L L V V V T D G E S H D G E

GAACTTCCAGCAGCGCTAAAGGCTGTGAGGCTGGCAGAGTGACACGTTATGGGATTGCGGTGAGACTTGATCAAGTCCA 7600
N F Q Q R . R P V R L A E . H V M G L R . D L I K S
G T S S A K G L . G W Q S D T L W D C G E T . S S P
E L P A A L K A C E A G R V T R Y G I A V R L D Q V Q

GTTGTTTTGTTTTGTGTTGTATCGTAT 7680
S C F V L C C I V C V C V C V C V C V C V C V Y V . Y
V L F C V V S C V C V C V C V C V C V C V C V C V I
L F C F V L Y R V C V C V C V C V C V C V C V C V I

GTGTGCATGCATCAGTGCACATACCATAGTGTGTATATGCGGGTCAGAGAACAACCTCAGATGTTGGTCTCCTCACCTTCCA 7760
V C H H Q C T Y H S V Y M R V R E Q P Q M L V L T F H
C A C I S A H T I V C I C G S E N N L R C W S S P S
C V H A S V H I F . C V Y A G Q R T T S D V G P H L P

TCTTGTTCAAAAGTATCTTCTTCACTTCGGCATACAATAAGCCAGATTAGCTGACCCACAAGTCTTGGGCGAGGTCT 7840
L V F N W I S C S L R H T I S O I S . P T S L G Q V
I L F Q T G Y L V H F G I Q . A R L A D P Q V L G R S
S C S K L D I L F T S A Y N K P D . L T H K S W A G L

TCTGTCTCAGCCTCCTGCTCTTGTGTTTGAGGCAATTCTGGAATTTACAGATAAGCTTGATATCGAATTCCTGACCCCGG 7920
F C L S L L S L G L R H S G I Y R . A . Y R I P A A R
S V S A S C L L V . G I L E F T D K L D I E F L O P G
L S Q P P V S W F E A F W N L Q I S L I S N S C S P

GGGATCCACTAGTTCTAGAGCGCGCCGCCACCAAGGGAG 7958
G I H . F . S G R H Q G S
G S T S S R A A A T K G
G D P L V L E R P P P R E

FIGURE 15f

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1 2 3 4

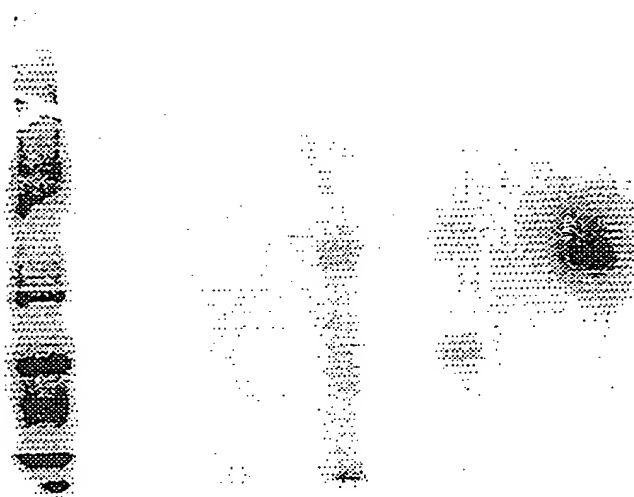


FIGURE 16

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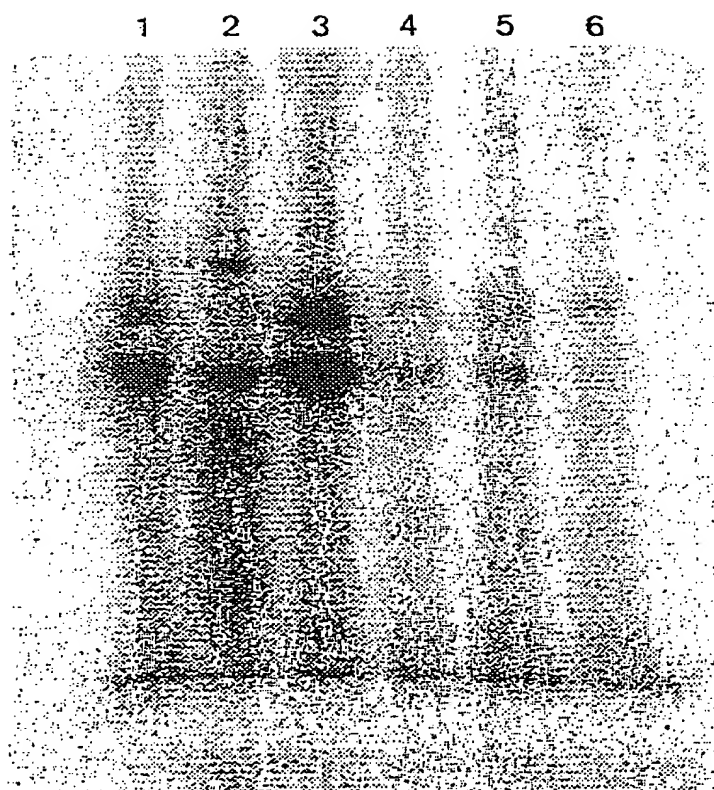


FIGURE 17